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Halo acids and DMSO: Electrophilic aromatic substitution at room temperature conditions

Pankaj Kulshrestha

HALO ACIDS AND DMSO: ELECTROPHILIC AROMATIC SUBSTITUTION AT ROOM TEMPERATURE CONDITIONS

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THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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[PANKAJ KULSHRESTHA]

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I would like to express my sincere gratitude and thanks to other members of my research committee - Prof. James C. Aumer, Director and Head of Department of Clinical Biochemistry program at RIT, NY and Dr. Robert H. Paine, Professor, Department of Chemistry and my teaching advisor for their invaluable guidance, constructive suggestions and support throughout my research work.

Lastly I would like to take this opportunity to thank almighty God, my parents, all my professors and my friends for their moral support and being there to help me whenever I needed their help.

Pankaj Kulshrestha

ABSTRACT

HALO ACIDS AND DMSO: ELECTROPHILIC AROMATIC SUBSTITUTION AT ROOM TEMPERATURE CONDITIONS

By Pankaj Kulshrestha under the guidance of Dr. James J. Worman

A simple, safe and cost effective experiment to demonstrate electrophilic aromatic substitution in the undergraduate laboratory can be accomplished by placing a small amount of N,N Dimethyl Aniline or a few crystals of Phenol in an NMR tube containing an appropriate level of deutero DMSO. After observation of the expanded proton NMR spectrum of the aromatic region, a few drops of concentrated HBr and HCl aqueous acids are added and the NMR spectrum recorded after one hour.

Observation of an A,A' B,B' typical four line pattern in the aromatic region of NMR spectrum is direct evidence for the halogenation on the aromatic ring at para position. Addition of gaseous hydrogen chloride provides a faster and cleaner reaction. A mechanism for the reaction, GC/MS data to support the NMR results, along with other examples are presented.

Application of the reaction for halogenation of complex biomolecules in an attempt to enhance their activity is plausible because room temperature experimental conditions should prevent structural degradation of sensitive molecules and produce fewer non-toxic byproducts.

INTRODUCTION

The objective of my research was to synthesize halogenated biomolecules in an attempt to enhance the activity against antibiotic resistant strains of bacteria and to reduce toxic byproducts of halogenation reactions in an undergraduate laboratory, thereby minimizing environmental pollution.

Current methods of aromatic halogenation involve the use of reagents and catalysts under experimental conditions which present a safety hazard when performed in an undergraduate teaching laboratory. In addition, the disposal of wastes are costly and definitely not benign to the environment.

Existing methods of halogenation involve the use of liquid bromine, chlorine gas and other hazardous halogenating reagents in the presence of catalysts (1,2). The methods employ drastic conditions which would destroy the structure of the biomolecules and perhaps decrease the pharmacological activity of the biomolecules, even causing them to be acutely toxic. The reaction conditions presently employed may also produce byproducts, many of which are hazardous to the environment (3,4).

Experimental conditions which on one hand involve halogenation of biomolecules safely with the formation of fewer toxic byproducts and on the other hand stereospecifically and

stereoselectively allow for the synthesis of biomolecules targeted to resistant microbial strains, would minimize environmental pollution and add knowledge to the field of medicinal chemistry.

Biomolecular target specificity can reduce the toxic ill effects and side effects on human or animal species and also prevent environmental pollution, which in turn may provide a safe living environment for other organisms in air, soil and water.

Safe disposal of the toxic byproducts of chemical reactions, in particular reactions involving halogenation, have been a matter of deep concern because byproducts have the potential to act as environmental pollutants. A part of this research focuses on the issue of trying to design safe and environmentally benign synthetic reactions for the research labs and for industry.

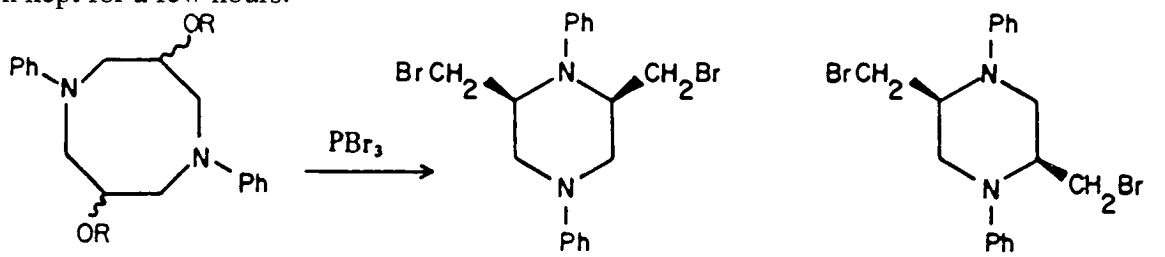
The design of such reactions promises the generation of stereoselective and stereospecific products and the safe disposal of unwanted byproducts, primarily by the use of haloacids in dimethyl sulfoxide under room temperature conditions. This same experimental design can be used in undergraduate laboratories to demonstrate halogenation reactions which can be accomplished with minimum waste and improved safety, thereby decreasing the risk of severe laboratory accidents.

EARLY INVESTIGATIONS

Before and during the 1960's and the 1970's electrophilic aromatic halogenation using liquid bromine/ferric bromide and chlorine gas/ferric chloride or other hazardous halogenating reagents and catalysts was not only studied in theory (1,2,5,6) but also included in laboratory courswork (7).

This important chemical reaction and its mechanism is taught in undergraduate coursework (8,9,10,11,12) and graduate coursework (13,14), but due to the use of expensive and hazardous reagents and unsafe experimental conditions, these experiments were removed from the undergraduate laboratory experimental curriculum (15,16).

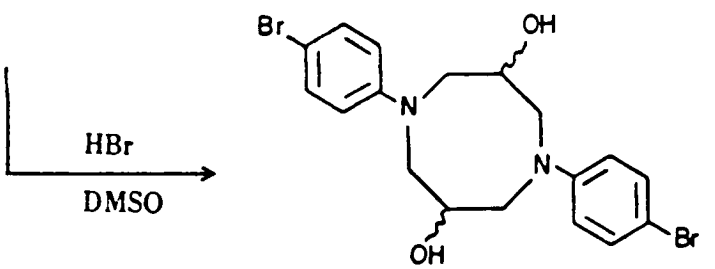
As early as 1970's (17,18) during the ongoing research (**scheme 1 below**) by Dr. James J.Worman, it was found that the dihydrobromide salt of 3,7 dimethoxy-1,5 diphenyloctahydro-1,5 diazocine, underwent halogenation in the presence of dimethyl sulfoxide in the NMR tube when kept for a few hours.



1 R = H
5 R = C-CH$_3$
O

2

3



4

Figure 1. below shows a typical four line pattern of the aromatic region of a para substituted aromatic system(1,5-Bis-(p-bromo phenyl)octahydro-3,7- dimethoxy-1,5-diazocine) – the compound which was formed during the ongoing researchwork done by Dr.Worman's research group, when dimethyl sulfoxide was added to the dihydrobromide salt of octahydro 3,7-dimethoxy- 1,5- diphenyl- 1,5- diazocine in an NMR tube.

Figure 1.

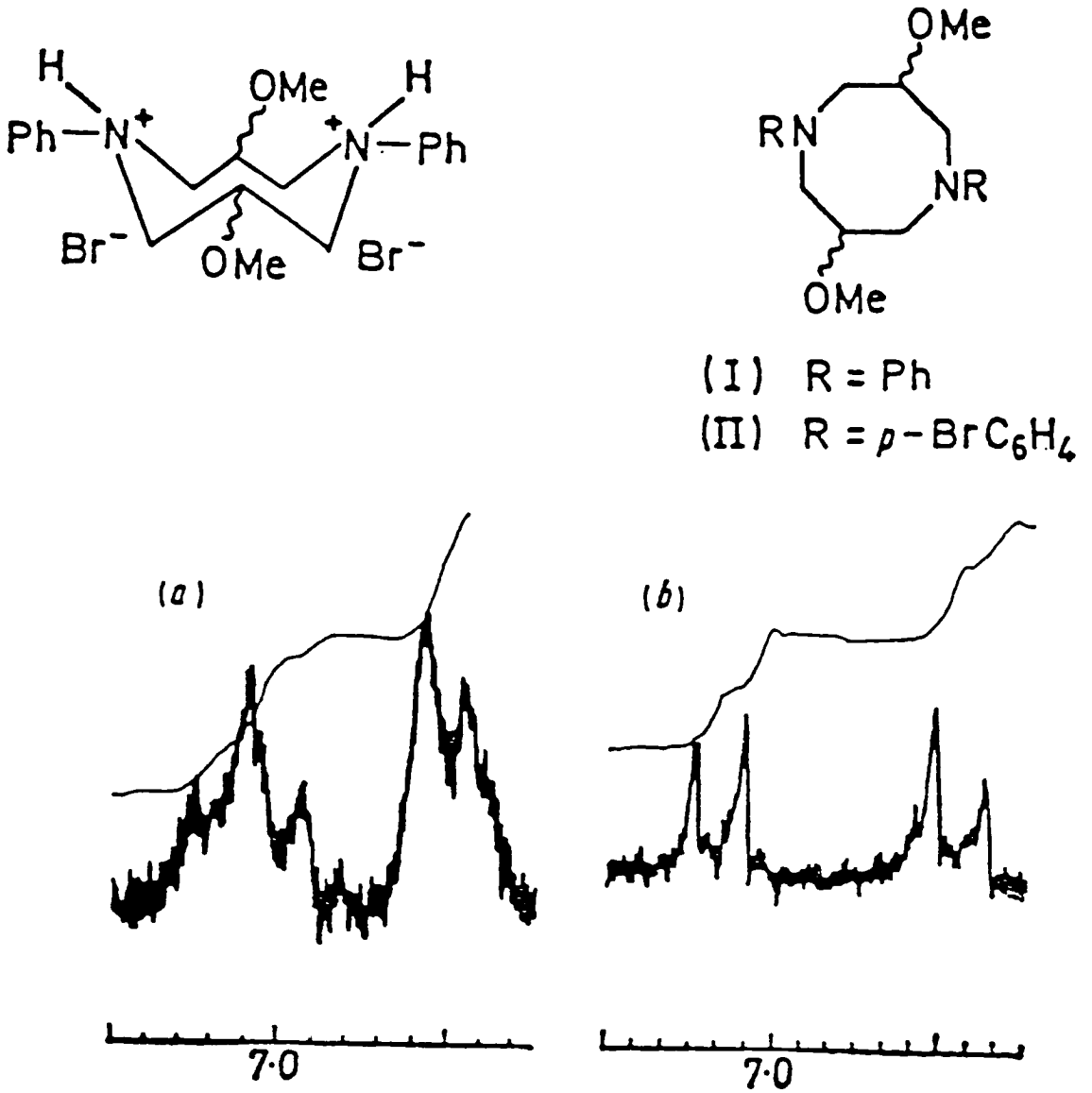
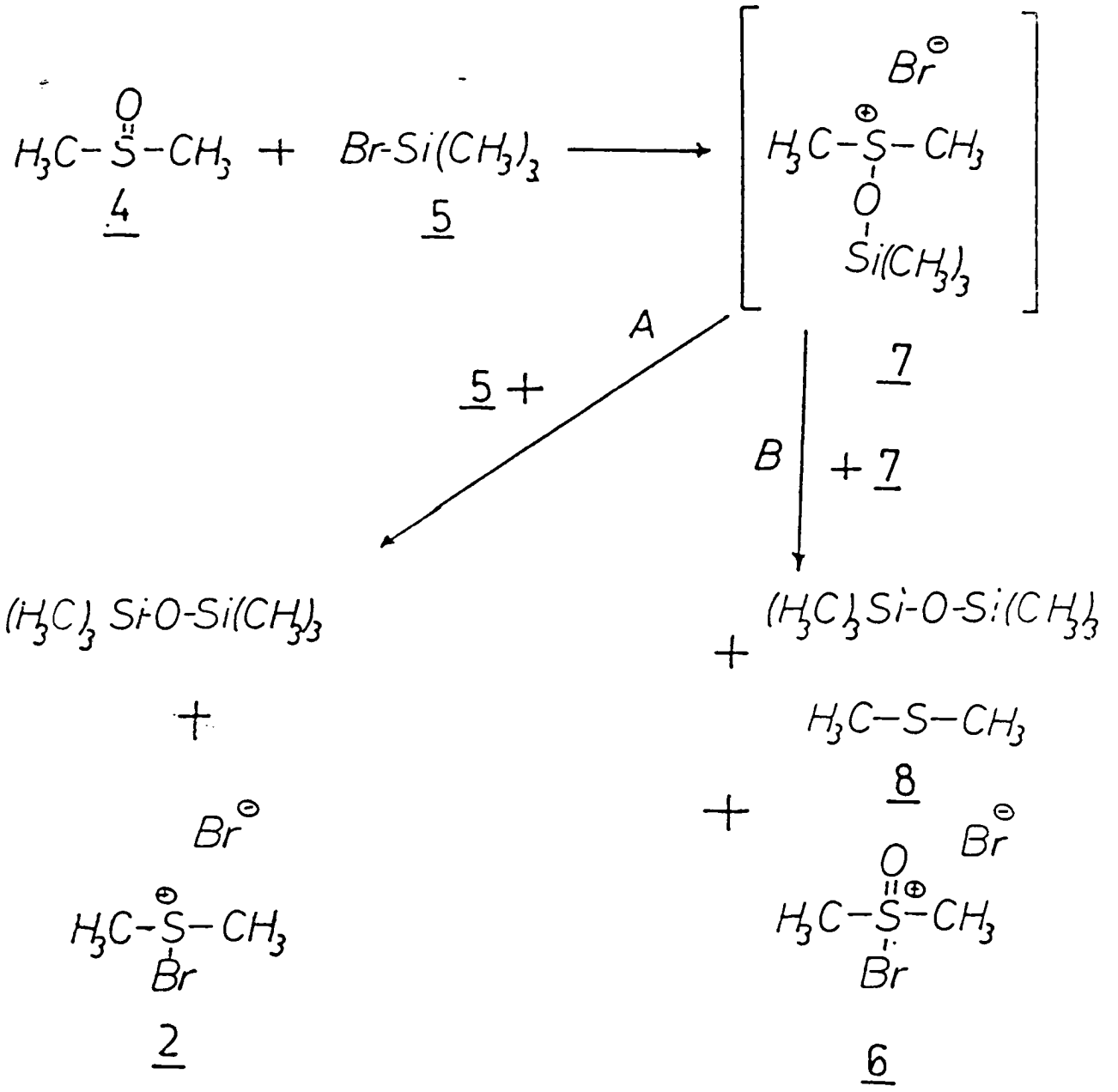
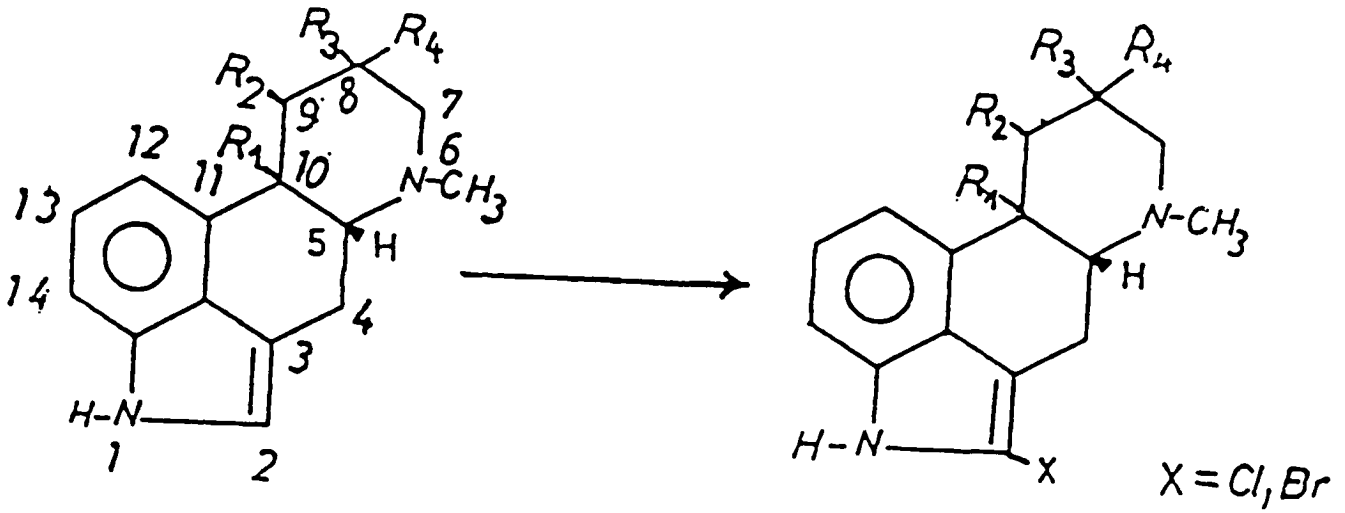


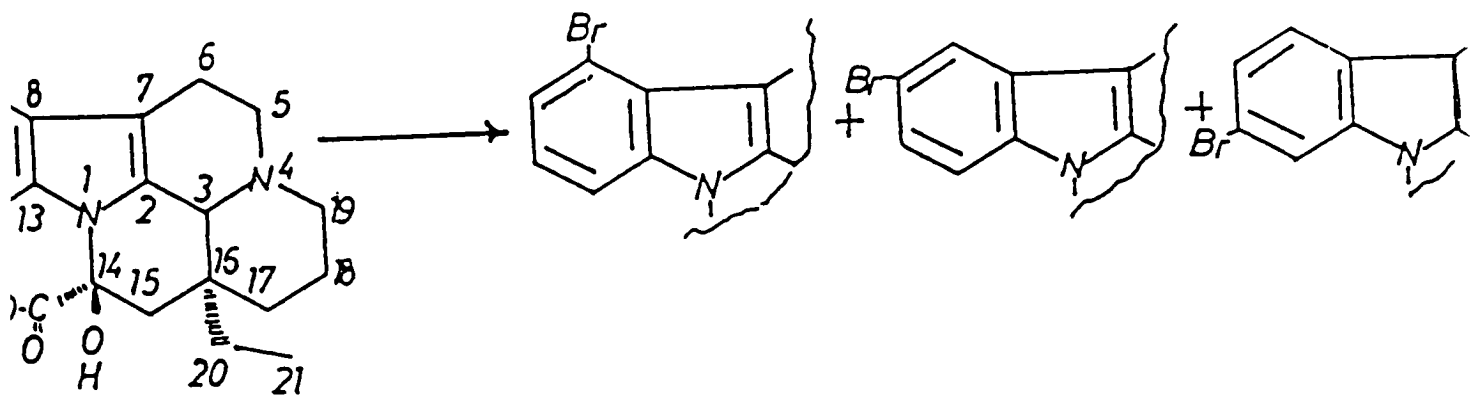
FIGURE 1 N.m.r. spectra of the dihydrobromide of octahydro-3,7-dimethoxy-1,5-diphenyl-1,5-diazocine (I) in dimethyl sulphoxide at room temperature; (*a*) aromatic-proton absorption immediately after dissolution of dihydrobromide in solvent; (*b*) same absorption after 8 h

Very little work was done in this area until 1989 when Dr.Megyeri Gabor and Dr. Tibor Keve of Hungary halogenated indole alkaloids using halodimethylsulfonium halogenids (compound no.2 in **scheme 2 below**) and halodimethylsulfoxonium halogenids (compound no.6 in **scheme 2 below**)(19).



These are only a few examples out of many reactions carried out by Dr.Gabor's research group which show that halogenation can take place not only at the vinyl position but also on the aromatic ring using previously stated conditions (**scheme 3 below**).





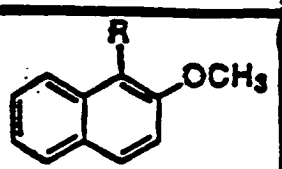
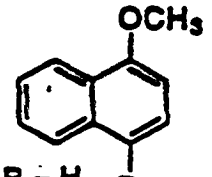
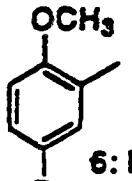
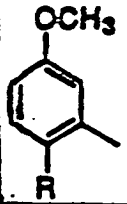
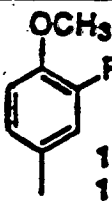
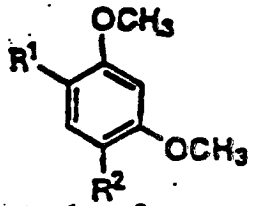
In 1997, pioneer work was done by Dr.George Majetich's research group when they presented a myriad of reaction conditions (**table1,2 and 3 below**)and procedures to specifically brominate various aromatic and heterocyclic compounds (20).

It is a distinct possibility that these conditions can be used to brominate aromatic macromolecules to give useful chemical compounds and produce few non-toxic byproducts, the reaction conditions are easy to follow and have the potential for reaction specificity, which could form targeted biomolecules. The utility of such halogenation conditions and reaction methodologies, which were not attempted earlier, were investigated.

Table 1

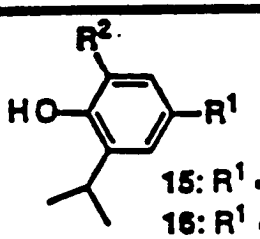
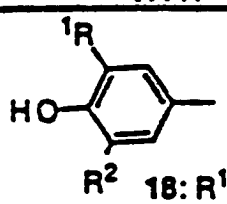
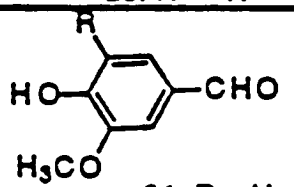
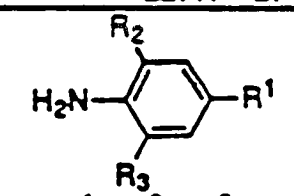
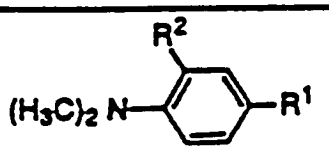
solvent	reaction condns	yield of 3 (%)	reaction completion
DMSO	2 h at rt	96	complete
acetonitrile	32 h at rt	84	trace unreacted 2
THF	1 h at reflux	trace	only unreacted 2
THF	24 h at reflux	99	complete
THF/AcOH (3:1)	2 h at rt	50	~50% complete
AcOH/DMSO (2:1)	<5 min	96	complete

Table 2

Substrate / Product	Percent Yield and Reaction Time Using Standard Conditions			
	HBr, DMSO "A"	HBr, AcOH, DMSO "B"	Br_2, CCl_4 "C"	Br_2, AcOH "D"
 2: R = H 3: R = Br	96% of 3 after 2-h	96% of 3 after 5-min	90% of 3 after 5-min	89% of 3 after 5-min
 4: R = H 5: R = Br	77% of 5 after 72-h	97% of 5 after 24-h	90% of 5 after 72-h	85%[a] of 5 after 72-h
 6: R = H 7: R = Br	82% of 7 after 12-h	86% of 7 after 12-h	75% of 7 after 5-min	75% of 7 after 2-h
 8: R = H 9: R = Br	91% of 9 after 12-h	90% of 9 after 12-h	68%[a] of 9 after 20-min	89%[a] of 9 after 20-min
 10: R = H 11: R = Br	77% of 11 after 72-h	75% of 11 after 72-h	74% of 11 after 20-min	91% of 11 after 5-min
 12: $R^1 = R^2 = H$ 13: $R^1 = H$; $R^2 = Br$ 14: $R^1 = R^2 = Br$	34% of 13 after 5-min 89% of 14 after 20-min	73% of 14 after 5-min 87% of 14 after 20-min	80%[a] of 14 after 5-min	77% of 14 after 5-min

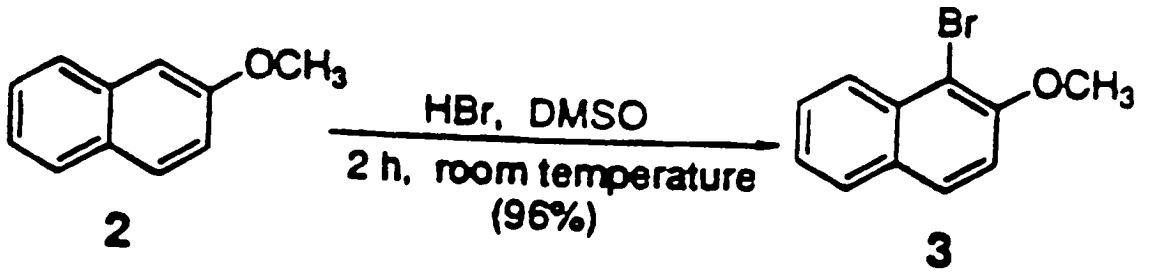
[a] Product contained inseparable impurities. The yield shown is based on analysis of the ^1H NMR spectrum.

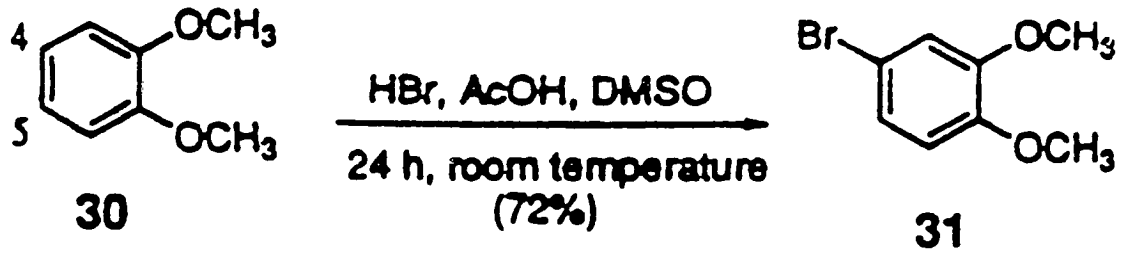
Table 3

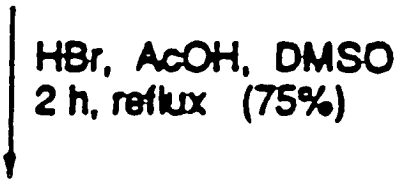
Substrate / Product	Percent Yield and Reaction Time Using Standard Conditions			
	HBr, DMSO "A"	HBr, AcOH, DMSO "B"	Br_2, CCl_4 "C"	Br_2, AcOH "D"
 15: $R^1 = R^2 = H$ 16: $R^1 = Br$; $R^2 = H$ 17: $R^1 = R^2 = Br$	90% of 16 after 24-h	91% of 16 after 5-min	23% of 16 and 64% of 17 after 5-min	63% of 16 and 31% of 17 after 5-min
 18: $R^1 = R^2 = H$ 19: $R^1 = Br$; $R^2 = H$ 20: $R^1 = R^2 = Br$	88% of 19 after 48-h	87% of 19 after 2-h	87% of 19 after 30-min	83% of 19 after 30-min; trace 20
 21: R = H 22: R = Br	72% of 22 after 12-h	57% of 22 after 12-h	48% of 22 after 24-h	80% of 22 after 20-min
 23: $R^1 = R^2 = R^3 = H$ 24: $R^1 = Br$; $R^2 = R^3 = H$ 25: $R^1 = R^2 = Br$; $R^3 = H$ 26: $R^1 = R^2 = R^3 = Br$	76% of 24 after 6-h	74% of 24 after 24-h	19% of 24; 33% of 25 and 30% of 26 after 5-min	21% of 24; 41% of 25 and 31% of 26 after 5-min
 27: $R^1 = R^2 = H$ 28: $R^1 = Br$; $R^2 = H$ 29: $R^1 = R^2 = Br$	43% of 28 after 24-h	42% of 28 after 24-h	60% of 28 after 5-min	68% of 28 and 29% of 29 after 5-min

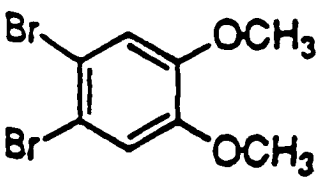
[a] Inseparable byproducts were observed. The yield shown is based on analysis of the ^1H NMR data.

Scheme 4 and 5 show a few examples of many reactions carried out by Dr.Majetich's research group. Scheme 5 shows that under room temperature halogenation conditions one position on the aromatic ring of Veratrole (compound 30 in **scheme 5**) is halogenated and under severe reflux halogenation conditions more than one position on the aromatic ring of the same compound is halogenated.









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EXPERIMENTAL WORK

The following experimental work was done in the Department of Chemistry at the Rochester Institute of Technology, Rochester, NY under the supervision of Dr.James J. Worman.

All nuclear magnetic resonance data were obtained on a 7 Tesla Bruker DRX – 300 300 MHz spectrometer. Deuterated dimethyl sulfoxide was used as the solvent for all samples.

All GC/MS data were obtained on a Hewlett Packard model HP190915S-936. The column used for the entire analysis was a capillary column (60.0mts * 250.0m * 0.25m in dimensions) tightly packed with HP-1MS-crosslinked Methyl Siloxane. Conditions employed were kept uniform during the entire analysis of samples. Uniform conditions used were constant flow of gas at the rate of 1ml/min and total flow being 33.4 ml/min under a total pressure of 35.23 psi, the temperature of the heater/injector was set at 250°C. The column was operated at a temperature range of 50°C-280°C. The temperature of the column was held at 50°C for one minute, then ramped at 20°C/min till 180°C then reached a final temperature of 280°C to which it was set at the rate 30°C/min and held for 2 minutes. Dimethyl Sulfoxide, Tetrahydrofuran and Methylene Chloride were used as diluting solvents during the GC/MS run of various samples.

Typical NMR and GC/MS spectra are shown in Appendix A and B respectively.

THREE PHASES OF MY EXPERIMENTAL WORK INCLUDE

PHASE I :- involves the use of NMR to determine the formation of para substituted halogenated compounds of simple aromatic systems.

PHASE II :- involves the use of GC/MS analysis to verify the NMR results and to determine the isomeric distribution of halogenated species formed.

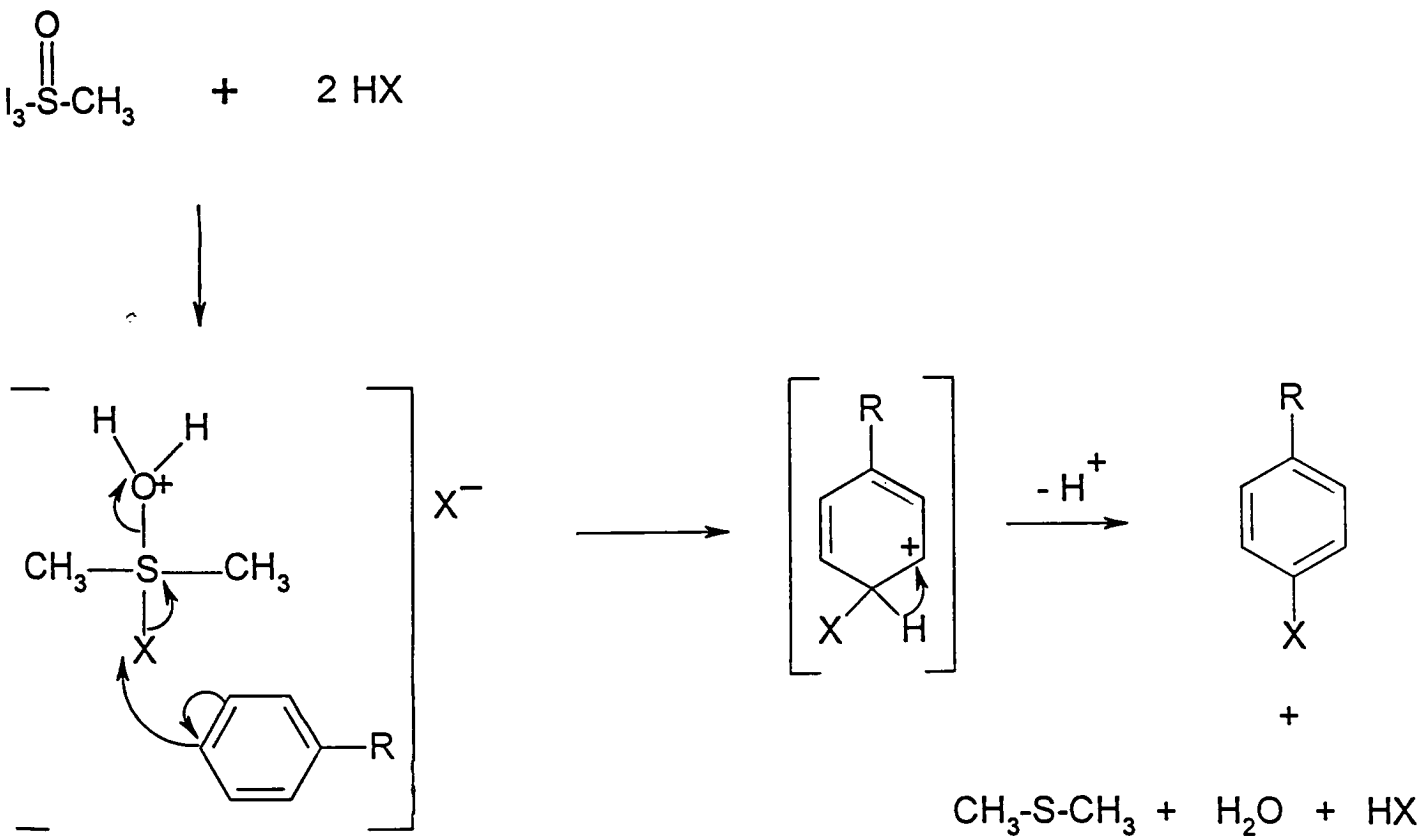
PHASE III :- involves the use of NMR and GC/MS analysis to determine the position of halogen substitution on the macro/biomolecules.

TWO POINT APPROACH UTILIZED IN THE RESEARCH

1) Use of room temperature conditions for halogenation.
2) Ensure safe halogenation of aromatic systems by utilizing novel but simple laboratory techniques.

REACTION MECHANISM

Two moles of a halo acid react with one mole of dimethyl sulfoxide. Halogen anion (X^-) of one mole of the halo acid attacks the sulfur of dimethyl sulfoxide and hydrogen cation H+ of the same halo acid attacks the oxygen of the dimethyl sulfoxide giving rise to a tetrahedral intermediate state. The hydrogen cation H+ of a second mole of the haloacid also attacks the oxygen of the hydroxy group to give a positive oxonium ion balanced by the halogen anion(X^-). The halogen attached to the sulfur of the tetrahedral intermediate attacks the para position of the aromatic ring of an aromatic system with the simultaneous in-situ formation of the para substituted halogenated aromatic system, water and dimethyl sulfide.

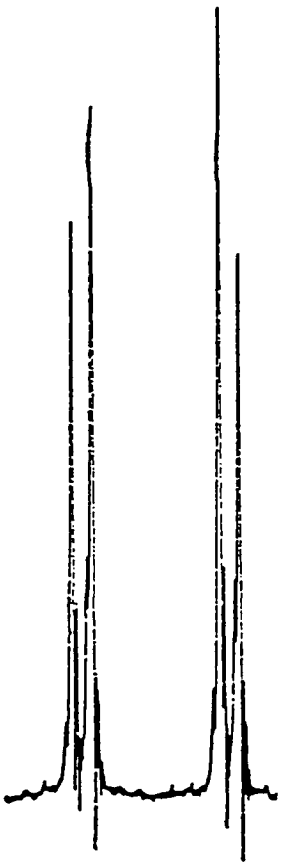


EXPERIMENTAL PROCEDURE

Aqueous Experimental Conditions:- To two drops of N,N-Dimethylaniline in an NMR tube was added 1 ml of deuterated dimethyl sulfoxide followed by the addition of 2-4 drops of concentrated haloacid and the solution was left at room temperature for one to two hours. Thereafter, the NMR and GC/MS analysis was performed to determine the in-situ formation of para substituted halogenated aromatic species and other isomers (Typical NMR resuits are shown in **figure 2.** on next page.)

Gaseous Experimental Conditions:- To two drops Anisole in an NMR tube was added 1 ml of deutero DMSO followed by passing gaseous HCl at a controlled rate of one gas bubble/second for 30 seconds and then the solution was heated in an oil bath at the temperature of 100°C for one hour. The solution was left to stand at room temperature for one hour, afterwhich NMR and GC/MS analysis was performed to observe the in-situ formation of para substituted halogenated aromatic species (Typical NMR results are shown in **figure 2.** on next page.)

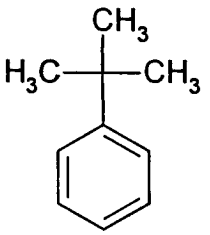
Figure 2. TYPICAL FOUR LINE PATTERN OF A PARA SUBSTITUTED AROMATIC SYSTEM WITH TWO DIFFERENT SUBSTITUENTS



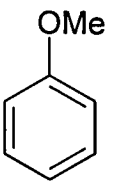
TYPICAL FOUR LINE PATTERN OF A PARA SUBSTITUTED AROMATIC SYSTEM WITH TWO DIFFERENT SUBSTITUENTS

EXPERIMENTAL PHASE I

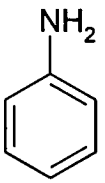
following aromatic systems were halogenated in an effort to establish environmentally benign room perature conditions for safe and simple halogenation experiments in an undergraduate laboratory. he same time a search was made to develop the best halogenating reagents under room temperature ditions which could be used for halogenation of biomolecules.



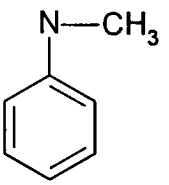
t-BUTYL BENZENE



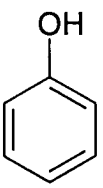
ANISOLE



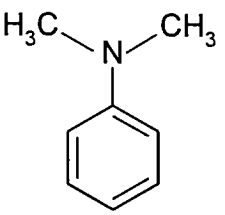
ANILINE



N METHYL ANILINE



PHENOL



N,N DIMETHYL ANILINE

Table 4 : LIST OF HALOGENATION REACTIONS AND NMR ANALYSIS RESULTS

LIST OF REACTIONS	NMR RESULTS
TERT.BUTYLBENZENE +CONC.HF +DMSO	- ve
TERT.BUTYLBENZENE +CONC.HCl +DMSO	- ve
TERT.BUTYLBENZENE +CONC.HBr +DMSO	- ve
TERT.BUTYLBENZENE +CONC.HI +DMSO	- ve
TERT.BUTYLBENZENE +GASEOUS HCl + DMSO	- ve
ANISOLE + CONC.HF + DMSO	- ve
ANISOLE + CONC.HCl + DMSO	- ve
ANISOLE + CONC.HBr + DMSO	- ve
ANISOLE + CONC.HI + DMSO	- ve
ANISOLE + GASEOUS HCl + DMSO (HEATED IN OIL BATH FOR 1 Hr.)	+ ve
ANILINE + CONC.HF + DMSO	- ve
ANILINE + CONC.HCl + DMSO	+ ve
ANILINE + CONC.HBr + DMSO	+ ve
ANILINE + CONC.HI + DMSO	- ve
ANILINE + GASEOUS HCl + DMSO	+ ve
N-METHYLANILINE + CONC. HF + DMSO	ve
N-METHYLANILINE + CONC.HCl + DMSO	+ ve
N-METHYLANILINE + CONC.HBr. + DMSO	+ ve
N-METHYLANILINE + CONC.HI + DMSO	- ve
N-METHYLANILINE + GASEOUS HCl + DMSO	+ ve
N,N-DIMETHYLANILINE + CONC. HF + DMSO	- ve
N,N-DIMETHYLANILINE + CONC. HCl + DMSO	+ ve
N,N-DIMETHYLANILINE + CONC. HBr + DMSO	+ ve

N,N-DIMETHYLANILINE + CONC. HI + DMSO	- ve
N,N-DIMETHYLANILINE + GASEOUS HCl + DMSO	+ ve
PHENOL + CONC.HF + DMSO	ve
PHENOL + CONC.HCl + DMSO	+ ve
PHENOL + CONC.HBr + DMSO	+ ve
PHENOL + CONC.HI + DMSO	- ve
PHENOL + GASEOUS HCl + DMSO	+ ve

+ ve means that typical four line pattern of a para sustituted aromatic system was seen during NMR analysis

- ve means that typical four line pattern of a para substituted aromatic system was not seen during NMR analysis

EXPERIMENTAL PHASE II

Table 5 : NMR RESULTS(PARA, META, ORTHO SUBSTIUTED HALOGENATED AROMATIC SYSTEMS)SUPPORTED BY GC/MS ANALYSIS

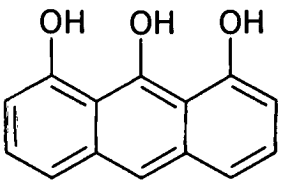
S.N.	LIST OF REACTIONS	PARA PRODUCT	META PRODUCT	ORTHO PRODUCT	% FORMED OUT OF ALL THE COMPOUNDS ELUTED
1.	TERT.BUTYL - BENZENE + CONC. HF + DMSO	- ve	-ve	-ve	-ve
2.	TERT.BUTYL - BENZENE + CONC. HCl + DMSO	- ve	- ve	- ve	- ve
3.	TERT.BUTYL - BENZENE + CONC. HBr + DMSO	- ve	- ve	- ve	- ve
4.	TERT.BUTYL - BENZENE + CONC. HI + DMSO	- ve	- ve	- ve	- ve
5.	TERT.BUTYL - BENZENE + GAS. HCl + DMSO	- ve	- ve	- ve	- ve
6.	ANISOLE + CONC. HF + DMSO	- ve	- ve	- ve	- ve
7.	ANISOLE + CONC. HCl + DMSO	- ve	- ve	- ve	- ve
8.	ANISOLE + CONC. HBr + DMSO	- ve	- ve	- ve	- ve
9.	ANISOLE + CONC. HI + DMSO	- ve	- ve	- ve	- ve
10.	ANISOLE + GAS. HCl + DMSO (HEATED IN OIL BATH FOR 2 Hrs.)	+ ve	- ve	+ ve	29.15% p-Chloroanisole 8.77% o-Chloroanisole
11.	ANILINE + CONC. HF + DMSO	- ve	- ve	- ve	- ve
12.	ANILINE + CONC. HCl + DMSO	+ ve	- ve	+ ve	26.36% p-Chloroaniline 4.85% o-Chloroaniline 2.13% 2,4-Dichloroaniline
13.	ANILINE + CONC. HBr + DMSO	+ ve	+ ve	+ ve	20.46% p-Bromoaniline 4.10% m-Bromoaniline 2.01% 2,4-Dibromoaniline

14.	**ANILINE + CONC. HI + DMSO**	- ve	- ve	- ve	- ve
15.	**ANILINE + GAS. HCl + DMSO**	+ ve	- ve	- ve	22.56% p-Chloroaniline 5.06% o-Chloroaniline 3.21% 2,4-Dichoroaniline
16.	**N-METHYL - ANILINE + CONC. HF + DMSO**	- ve	- ve	-ve	-ve
17.	**N-METHYL - ANILINE + CONC. HCl + DMSO**	+ ve	+ ve	+ ve	38.75% p-Chloro-N-methyl Aniline 9.25% m-Chloro-N-methyl Aniline 0.46% o-Chloro-N-methyl Aniline 1.19% 2,4-Dichloro-N-methyl Aniline
18.	**N-METHYL - ANILINE + CONC. HBr + DMSO**	+ ve	- ve	- ve	58.57% p-Bromo-N-methyl Aniline 5.01% 2,4-Dibromo-N-methyl Aniline
19.	**N-METHYL - ANILINE + CONC. HI + DMSO**	- ve	- ve	- ve	- ve
20.	**N-METHYL - ANILINE + GAS. HCl + DMSO**	+ ve	+ ve	+ ve	35.05% p-Chloro-N-methyl Aniline 6.23% m-Chloro-N-methyl Aniline 1.08% o-Chloro-N-methyl Aniline 0.77% 2,4-Dichloro-N-methyl Aniline
21.	**N,N-DIMETHYL - ANILINE + CONC. HF + DMSO**	- ve	- ve	- ve	- ve
22.	**N,N-DIMETHYL - ANILINE + CONC. HCl +DMSO**	+ ve	- ve	- ve	15.03% p-Chloro-N,N-dimethyl Aniline
23.	**N,N-DIMETHYL - ANILINE + CONC. HBr +DMSO**	+ ve	ve	- ve	60.32% p-Bromo-N,N-dimethyl Aniline
24.	**N,N-DIMETHYL - ANILINE + CONC. HI + DMSO**	- ve	- ve	- ve	- ve

25.	**N,N DIMETHYL - ANILINE + GAS. HCl + DMSO**	+ ve	- ve	- ve	17.78% p-Chloro-N,N-dimethyl Aniline
26.	**PHENOL + CONC. HF + DMSO**	- ve	- ve	- ve	- ve
27.	**PHENOL + CONC. HCl + DMSO**	+ ve	- ve	- ve	4.57% p-Chloro phenol
28.	**PHENOL + CONC. HBr + DMSO (2 DIFFERENT GC/MS RESULTS)**	A) + ve B) + ve	+ ve + ve	- ve + ve	12.86% p- & m-Bromo phenol in equal quantities 15.39% p-Bromo phenol 11.37% m-Bromo phenol 7.01% o-Bromo phenol
29.	**PHENOL + CONC. HI + DMSO**	- ve	- ve	- ve	- ve
30.	**PHENOL + GAS. HCl. + DMSO**	+ ve	- ve	- ve	5.03% p-Chloro phenol

EXPERIMENTAL PHASE III

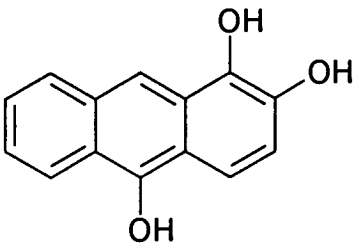
The following macro/biomolecules were selected for halogenation after thorough study of the Merck Index (21) and other organic medicinal chemistry books (22, 23, 24). They were halogenated with a possibility of enhancing their activity and forming a minimal quantity of toxic byproducts.



DITHRANOL

1,8,9 ANTHRACENETRIOL

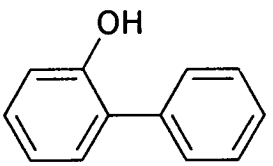
USED IN DERMATOLOGIC PREPARATIONS AND ANTIFUNGAL PREPARATIONS



ANTHRAROBIN

1,2,10 ANTHRACENETRIOL

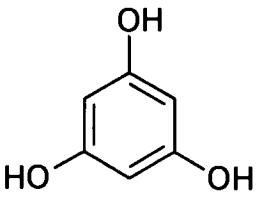
USED AS PARASITICIDE



O- PHENYL PHENOL (DOWICIDE I)

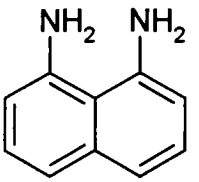
[1,1'-BIPHENYL] 2 - OL

USED IN GERMICIDE AND FUNGICIDE PREPARATIONS
AND IN COMMERCIAL RUBBER INDUSTRY



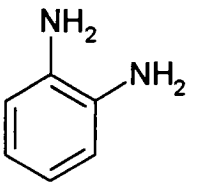
PHLOROGLUCINOL (1,3,5 BENZENETRIOL)

USED AS AN ANTISPASMODIC



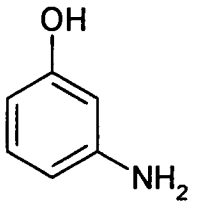
1,8 DIAMINO NAPTHALENE

USED AS AN ANTIOXIDANT FOR LUBRICATING OILS AND FOR THE DETECTION OF SELENIUM AND NITRITES



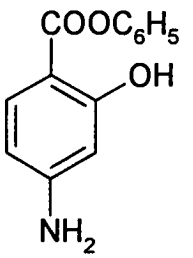
O- PHENYLENEDIAMINE(1,2- BENZENEDIAMINE)

USED IN THE MANUFACTURE OF DYES



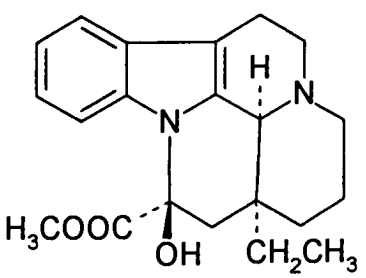
3 AMINO PHENOL

USED IN THE MANUFACTURE OF ANTITUBERCLAR AGENT P- AMINO SALICYLIC ACID(PAS)



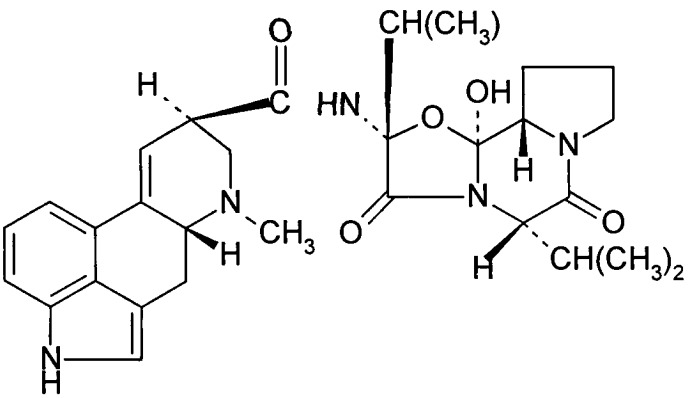
PHENYL AMINO SALICYLATE (PHENYL PAS)

USED AS AN ANTIBACTERIAL (TUBERCULOSTATIC)



VINCAMINE

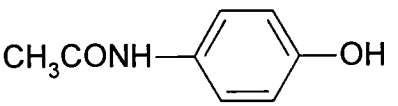
USED AS VASODILATOR



ERGOCORNINE

USED AS VASOCONSTRICTOR (SPECIFIC IN MIGRAINE).

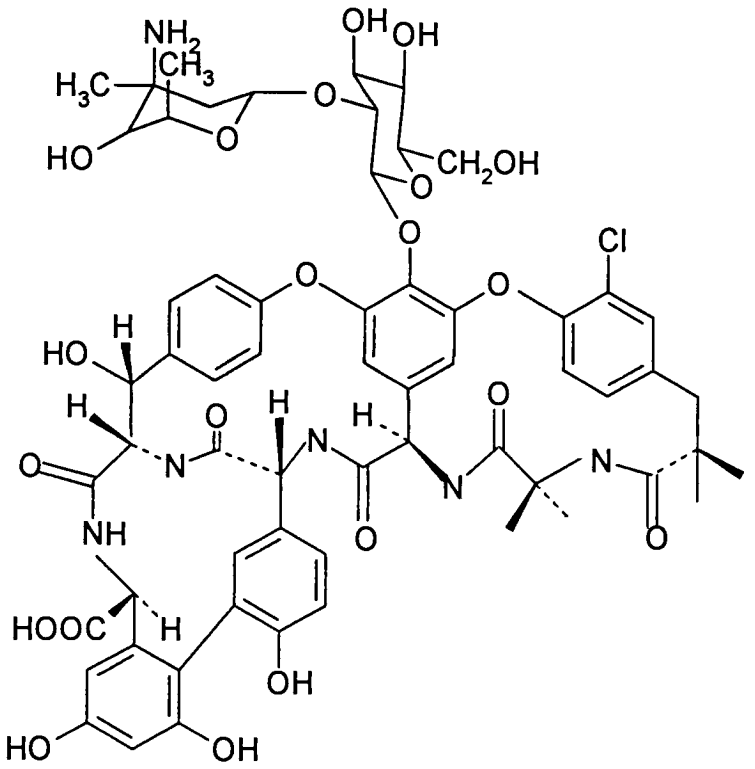
IT HAS BEEN USED AS AN OXYTOCIC IN VETERINARY MEDICINE



4 ACETAMIDOPHENOL

USED AS AN ANTIPYRETIC AND ANALGESIC

IT HAS BEEN USED COMMERCIALLY FOR THE MANUFACTURE OF AZO DYES AND PHOTOGRAPHIC CHEMICALS



VANCOMYCIN (VANCOCIN)

ANTIBIOTIC SUBSTANCE PRODUCED BY STREPTOMYCES ORIENTALIS FROM INDONESIAN AND INDIAN SOIL.

USED AS AN ANTIBACTERIAL

TABLE 6 : LIST OF HALOGENATION REACTIONS WITH MACRO/BIOMOLECULES AND NMR ANALYSIS RESULTS

LIST OF REACTIONS	NMR RESULTS
DITHRANOL + CONC. HCl + DMSO	UNINTERPRETABLE
DITHRANOL + CONC. HBr + DMSO	UNINTERPRETABLE
ANTHRAROBIN + CONC. HCl + DMSO	UNINTERPRETABLE
ANTHRAROBIN + CONC. HBr + DMSO	UNINTERPRETABLE
O- PHENYLPHENOL + CONC. HCl + DMSO	UNINTERPRETABLE
O- PHENYLPHENOL + CONC. HBr + DMSO	UNINTERPRETABLE
PHLOROGLUCINOL + CONC. HCl + DMSO	UNINTERPRETABLE
PHLOROGLUCINOL + CONC. HBr + DMSO	UNINTERPRETABLE
1,8 DIAMINONAPTHALENE + CONC. HCl + DMSO	UNINTERPRETABLE
1,8 DIAMINONAPTHALENE + CONC. HBr + DMSO	UNINTERPRETABLE
O- PHENYLENEDIAMINE + CONC. HCl + DMSO	UNINTERPRETABLE
O- PHENYLENEDIAMINE + CONC. HBr + DMSO	UNINTERPRETABLE
3 AMINOPHENOL + CONC. HCl + DMSO	UNINTERPRETABLE
3 AMINOPHENOL + CONC. HBr + DMSO	UNINTERPRETABLE
PHENYLAMINO SALICYLATE + CONC. HCl + DMSO	UNINTERPRETABLE
PHENYLAMINO SALICYLATE + CONC. HBr + DMSO	UNINTERPRETABLE
4 ACETAMIDOPHENOL + CONC. HCl + DMSO	UNINTERPRETABLE
4 ACETAMIDOPHENOL + CONC. HBr + DMSO	UNINTERPRETABLE
VINCAMINE + CONC.HCl + DMSO	UNINTERPRETABLE
VINCAMINE + CONC. HBr + DMSO	UNINTERPRETABLE

ERGOCORNINE + CONC. HCl + DMSO	UNINTERPRETABLE
ERGOCORNINE + CONC. HBr + DMSO	UNINTERPRETABLE
VANCOMYCIN + CONC. HCl +DMSO	UNINTERPRETABLE
VANCOMYCIN + CONC. HBr + DMSO	UNINTERPRETABLE

TABLE 7 : GC/MS ANALYSIS OF HALOGENATED PRODUCTS FORMED AS A RESULT OF HALOGENATION REACTIONS OF MACRO/BIOMOLECULES USING THE SAME CONDITIONS

S.N	LIST OF REACTIONS	HALOGENATED PRODUCT FORMED	PERCENTAGE PRODUCT FORMED
1.	DITHRANOL + CONC. HCl + DMSO	- ve	- ve
2.	DITHRANOL + CONC. HBr + DMSO	- ve	- ve
3.	ANTHRAROBIN + CONC. HCl + DMSO	- ve	- ve
4.	ANTHRAROBIN + CONC HBr + DMSO	- ve	- ve
5.	O- PHENYLPHENOL + CONC. HCl + DMSO	A) 5-Chloro-o-Phenyl phenol B) 3-Chloro-o-Phenyl phenol C) 2,2',3,4,5-pentaChloro-o-Phenyl phenol	3.57% 2.08% 0.4%
6.	O- PHENYLPHENOL + CONC. HBr + DMSO	A) 3,5-Dibromo-o-Phenyl phenol B) Bromophenoxy-Benzene	31.38% 3.78%
7.	PHLOROGLUCINOL + CONC. HCl + DMSO	- ve	- ve
8.	PHLOROGLUCINOL + CONC. HBr + DMSO	- ve	- ve
9.	1,8 DIAMINO - NAPTHALENE + CONC. HCl + DMSO	- ve	- ve
10.	1,8 DIAMINO - NAPTHALENE + CONC. HBr + DMSO	- ve	- ve
11.	O-PHENYLENEDIAMINE + CONC.HCl + DMSO	- ve	- ve
12.	O-PHENYLENEDIAMINE + CONC.HBr + DMSO	- ve	- ve
13.	3 AMINOPHENOL +CONC. HCl + DMSO	2,4-Dichloro 3-amino phenol	4.00%
14.	3 AMINOPHENOL + CONC. HBr + DMSO	2,4-Dibromo 3-amino phenol	18.35%
15.	PHENYLAMINO - SALICYLATE + CONC. HCl + DMSO	- ve	- ve

16.	**PHENYLAMINO - SALICYLATE + CONC. HBr + DMSO**	- ve	- ve
17.	**4 ACETAMIDOPHENOL + CONC. HBr + DMSO**	- ve	- ve
18.	**4 ACETAMIDOPHENOL + CONC. HCl + DMSO**	- ve	- ve
19.	**VINCAMINE + CONC. HCl + DMSO**	ve	- ve
20.	**VINCAMINE + CONC. HBr + DMSO**	- ve	- ve
21.	**ERGOCORNINE + CONC. HCl + DMSO**	- ve	- ve
22.	**ERGOCORNINE + CONC. HBr + DMSO**	- ve	- ve
23.	**VANCOMYCIN + CONC. HCl + DMSO**	- ve	- ve
24.	**VANCOMYCIN + CONC. HBr + DMSO**	- ve	- ve

RESULTS AND DISCUSSION

SIGNIFICANCE OF THE RESULTS ACHIEVED:-

1) Environmentally benign conditions were established for safe and simple halogenation experiments in the undergraduate laboratory

2) NMR and GC/MS analysis were used to verify the formation of specific isomers

POTENTIAL USE:-

1) Synthesis of halogenated biomolecules sensitive to pathogenic microbes in an attempt to enhance their bioactivity and reduce toxicity.

2) Minimizing environmental pollution, lessening hazards and ensuring safe disposal of waste products and toxic byproducts specifically to demonstrate electrophilic aromatic substitution in the undergraduate laboratory.

CONCLUSION

Aqueous HBr and HCl in the presence of DMSO prove to be good halogenating reagents of aromatic systems in the reactions previously described. Aqueous HI shows very little or no reactivity at all even though it is a stronger acid and should protonate the DMSO more effectively. Aqueous HF was also used, but there was no evidence that a reaction took place. This would be expected because fluorine does not like to be even partially positive, which is a necessity for electrophilic aromatic substitution. Gaseous HCl has almost the same reactivity as aqueous HCl. The only advantage in using gaseous HCl over aqueous HCl is that water interferes during NMR or GC/MS analysis of the reaction samples. The disadvantage in using gaseous HCl is that it is hazardous to use gas cylinders in undergraduate laboratories which besides being expensive, can pose a great safety hazard, cause environmental pollution and lead to unsafe handling problems.

The NMR data were uninterpretable for the reactions of macro/biomolecules in the presence aqueous acids and DMSO, because it seemed that water present in haloacids and deuterated DMSO(which absorbs water readily) was interfering with the final results, and the expected NMR pattern was not showing up clearly in the final results. Gaseous HCl was used for some of the reactions instead of aqueous HCl acid, but again because of interference of water present in DMSO (which appeared as a dominant peak in the NMR spectrum) and also

because of the complex aromatic region of most of these macro/biomolecules, the NMR spectrum was difficult to interpret.

Since all macro/biomolecules selected had –OH and $-NH_2$ as activating groups, and conditions for halogenation were also set according to those used for halogenating simple aromatic systems, it was assumed that some kind of halogenation reaction is taking place in the NMR tube even though NMR patterns were uninterpretable. Most of the reactions with macro/biomolecules did not show any positive GC/MS results. In some cases it was concluded that since most of these macromolecles have complex chemical structures with polar and non-polar groups and high boiling points, they tend to stick to the methyl siloxane GC/MS column and do not elute. This can be supported by the fact that even the parent molecule of these macro/biomolecules did not elute from the column. In some cases, as with Vancomycin and Phenyl Amino Salicylate, fragments of the molecules which were halogenated eluted from the column. It was inferred that halogenation might be taking place on the complex aromatic structure of these molecules, but since they did not elute as whole molecules during GC/MS analysis the above statement cannot be supported by any kind of evidence. Molecules like O-Phenyl phenol and 3-Amino phenol showed GC/MS evidence of halogenation when their halogenated products were successfully eluted from the GC/MS column by using a different eluting solvent i.e. CH_2Cl_2 rather than regular DMSO or THF. From this, a conclusion can be drawn that infact some macro/biomolecules can be halogenated using the conditions established for halogenation. Slight modification of the

halogenation conditions used and the use of suitable diluting and eluting solvents for analysis could be useful.

During GC/MS analysis, the percentage of halogenated products formed from all the products eluted from the GC/MS column and reported in tables 5 and 7 are not in very high yields. Many of the products could have been obtained from previous analysis and eluted with the solvents used in our GC/MS method of analysis. Isomer percentages were based on the area of individual isomers divided by the total area observed for all the products eluting from the GC/MS column.

Whatever the limitations, it can be successfully concluded from extensive halogenation reactions with simple aromatic systems that the reactions of haloacids with DMSO can be used to demonstrate electrophilc aromatic substitution in an undergraduate laboratory.

Similar conditions with slight modifications can be used to halogenate some bio/macromolecules.

REFERENCES

1. De la Mare, P.B.D., Ed., Electrophilic Halogenation : Reaction Pathways Involving Attack by Electrophilic Halogens on Unsaturated Compounds, Cambridge University Press, London (1976).
2. Norman, R.O.C., Taylor, R., Electrophilic Substitution in Benzenoid Compounds, Elseiver Publishing Company (1965)
3. Smeets, J., Ecotoxicol Environ Safety: New Challenges to Ecotoxicology (1979), 3:116
4. Gosselin, R.E. et al, Clinical Toxicology of Commercial Products, 4th Edition, Williams and Wilkins Publishers (1976)
5. House, H.O., Modern Synthetic Reactions, 2nd Edition, W.A. Benjamin, Inc. Publisher (1972)
6. Stacy, G.W., Organic Chemistry: a background for life Sciences, Harper and Row Publishers (1975)
7. Mackenzie, Charles A., Experimental Organic Chemistry, Prentice Hall, Inc. Publisher (1967)
8. Solomons, T.W.G., Fryhle, C.B., Organic Chemistry, 7th Edition, John Wiley & Sons, Inc. Publishers (1998)
9. Schmid, G.H., Organic Chemistry, Mosby Publishers (1996)
10. Vollhardt, K.P.C., Schore, N.E., W.H. Freeman and Company Publishers (1994)
11. Fox, M.A., Whitesell, J.K., Organic Chemistry, 2nd Edition, Jones and Bartlett Publishers (1997)
12. Engel, R., Baker, D.A., Organic Chemistry, West Publishing Company (1992)

13. Morrison, R.T., Boyd, N.R., Organic Chemistry, 6th Edition, Prentice Hall of India Pvt.Ltd. (1994)

14. Carey, F.A., Sundberg, R.J., Advanced Organic Chemistry, 3rd Edition, Plenum Press (1990)

15. Williamson, K.L., Macroscale and Microscale Organic Experiments, 3rd Edition, Houghton Mifflin Publishing Company (1999)

16. Linstromberg, W.W., Baumgarten, H.E., Organic Experiments, 6th Edition, D.C. Heath and Company (1987)

17. Worman, J.J., Kub, M.E., Pearson, M., *J.C.S. Perkin I*, 1209 (1972) and references there in.

18. Worman, J.J., Peters, C.Y., *J.Heterocyclic Chem.*, 14, 769 (1977) and references there in.

19. Megyeri, G., Keve,T., *Synthetic Commun.*, 19, 3415 (1989) and references there in.

20. Majetich, G., Hicks, R., *J.Org.Chem.*, 62, 4321 (1997) and references there in.

21. Windholz, M., Budavari, S., Stroumtsos, L.Y., Fertig, M.N., The Merck Index, 9th Edition, Merck & Co., Inc. Publisher (1976)

22. Lednicer, D., Mitschner, L.A., The Organic Chemistry of Drug Synthesis, Vol. 1,2 & 3 A Wiley - Interscience Publication (1980)

23. Burger's Medicinal Chemistry, Part I, II, III, 4th Edition, Edited by Wolff, M.E., A Wiley - Interscience Publication (1980)

24. Burger's Medicinal Chemistry & Drug Discovery, Vol. 1, 2, 3, 4 & 5, 5th Edition, Edited by Wolff, M.E., A Wiley - Interscience Publication (1997)

APPENDIX A

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APPENDIX B

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APPENDIX C

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APPENDIX D

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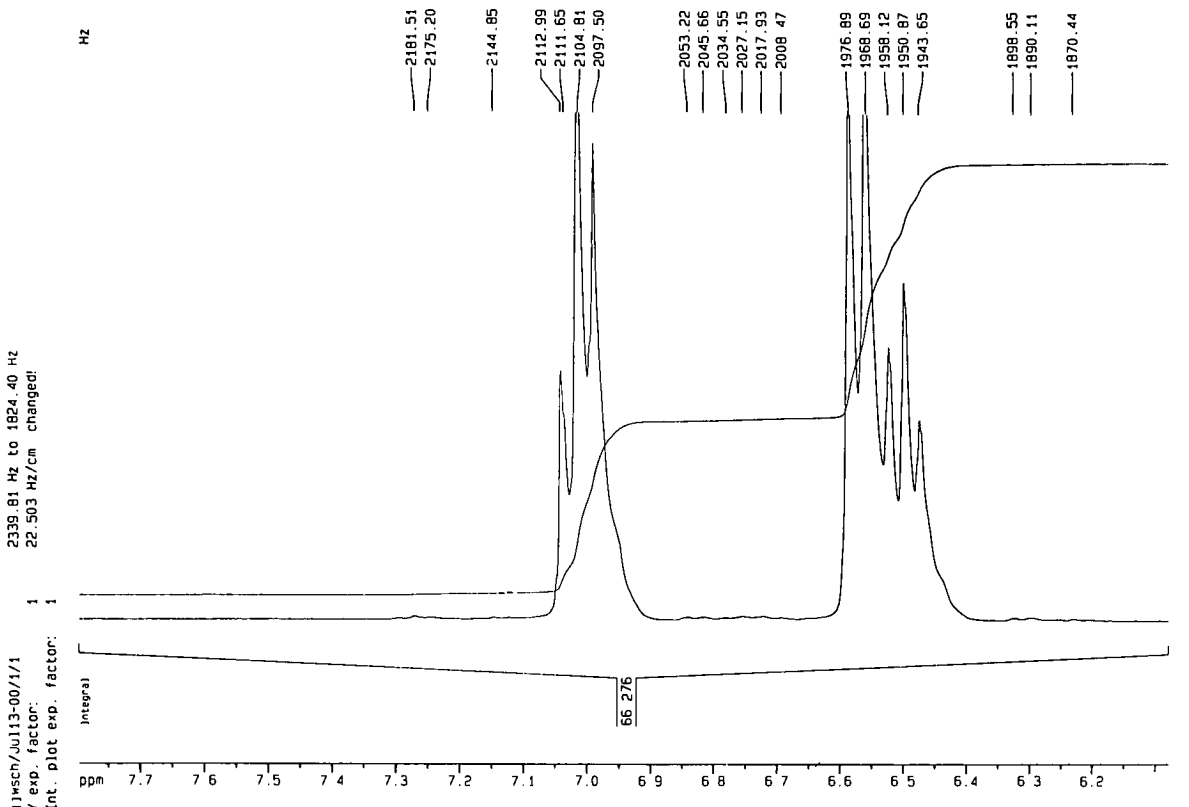
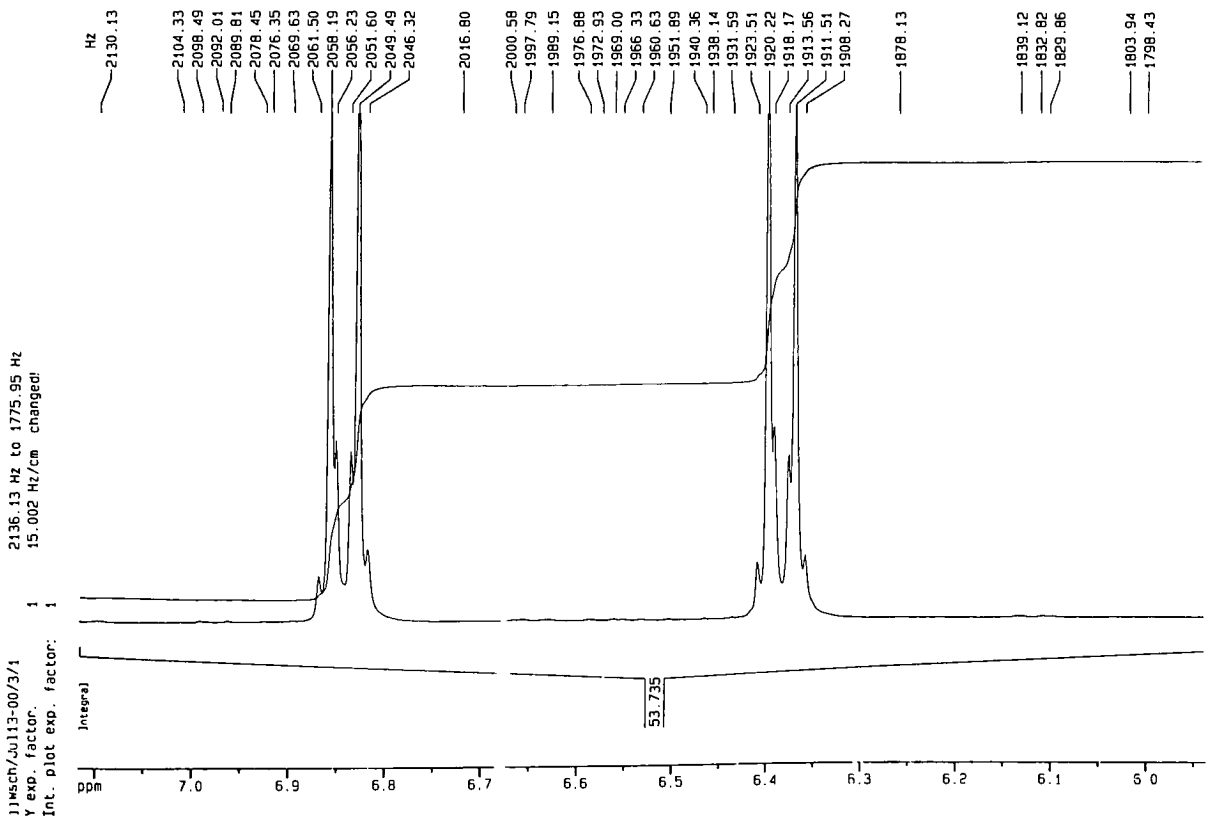
APPENDIX A

Spectrum 1.



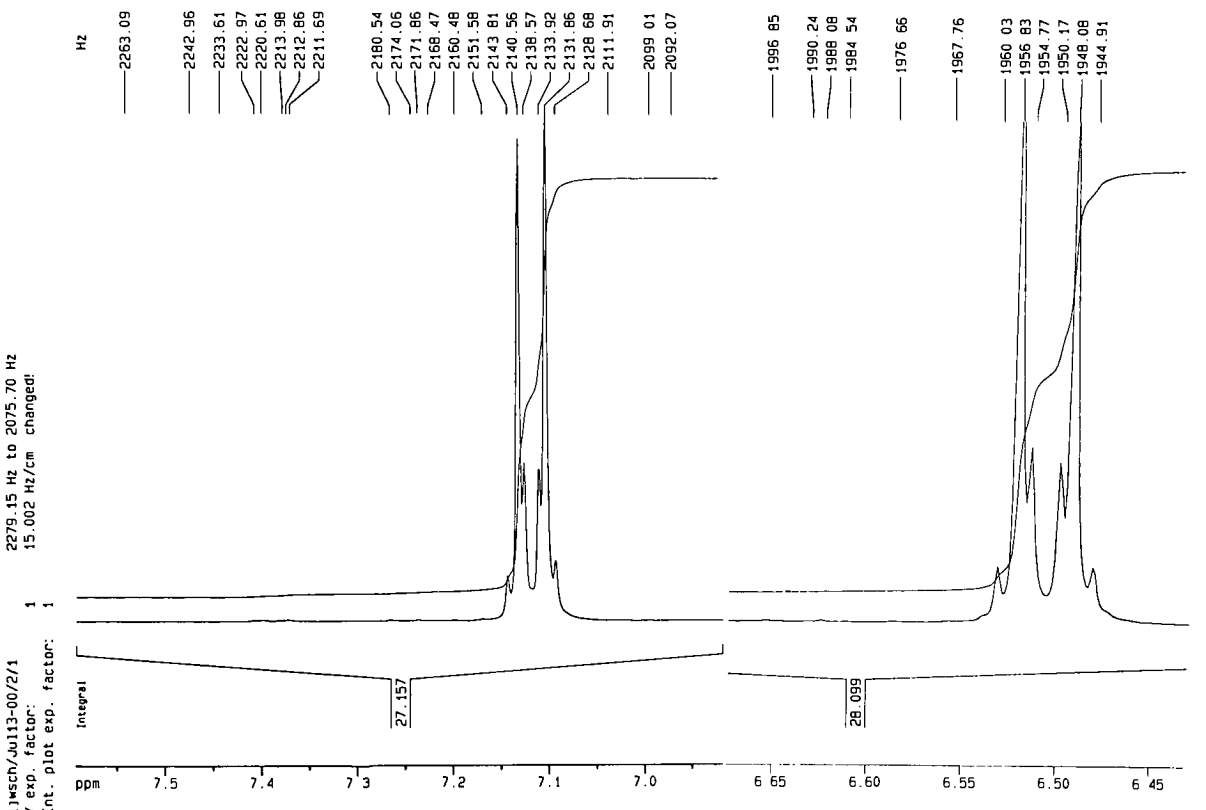
ANILINE (STANDARD) + DMSO

Spectrum 2.



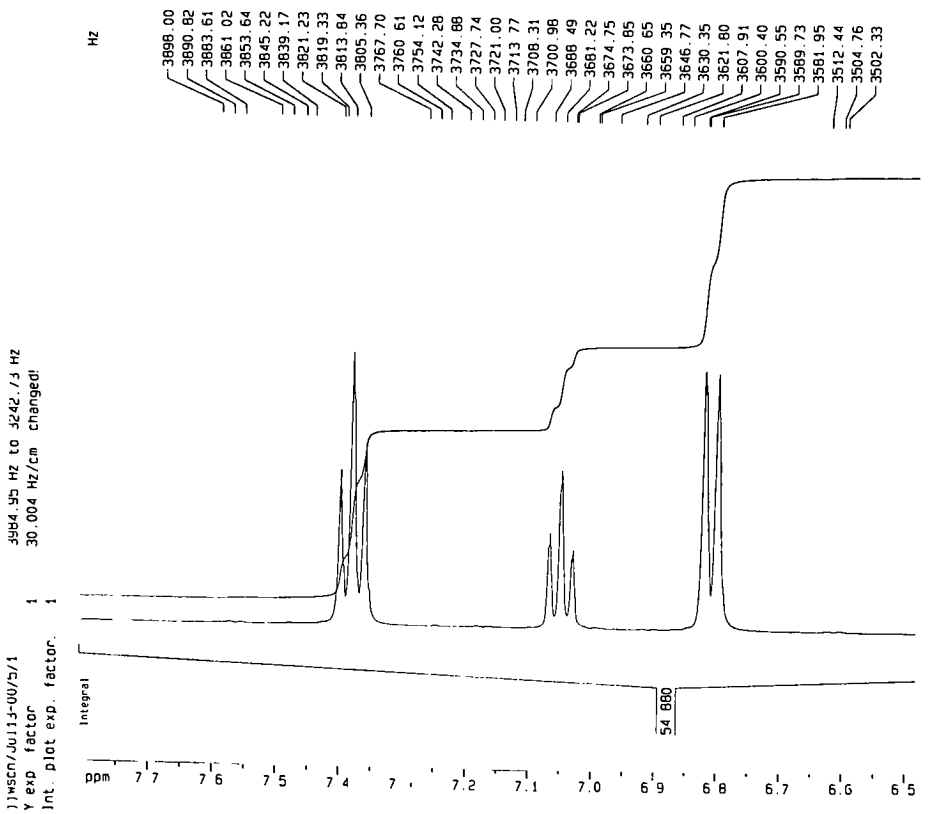
ANILINE + AQUEOUS HCl + DMSO

Spectrum 3.



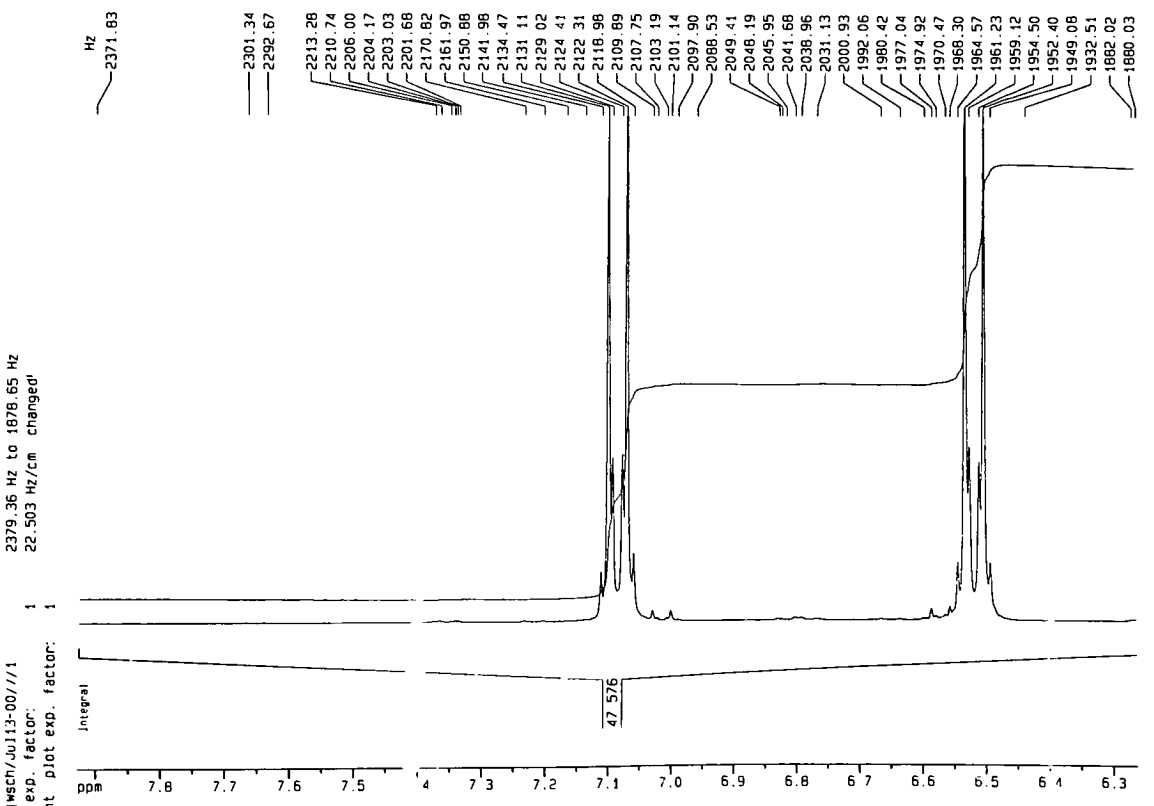
ANILINE + AQUEOUS HBr + DMSO

Spectrum 4.



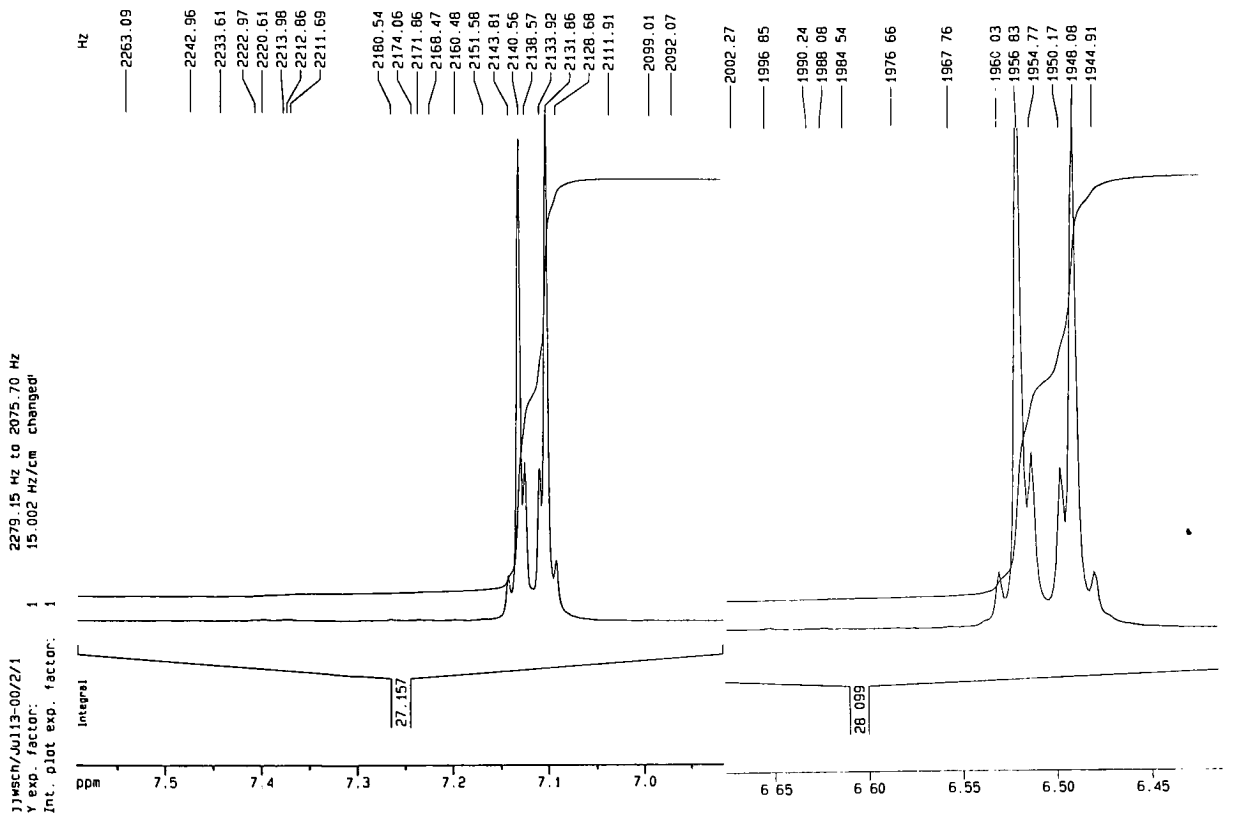
N METHYL ANILINE (STANDARD) + DMSO

Spectrum 5.



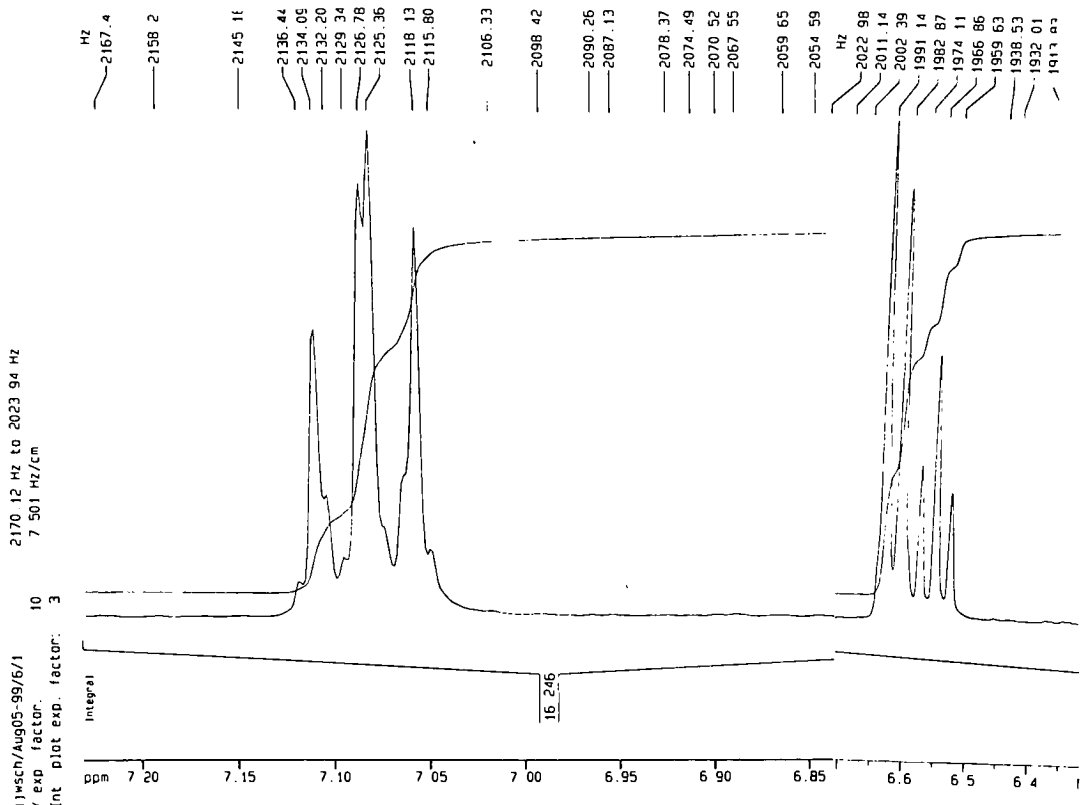
N METHYL ANILINE + AQUEOUS HCl + DMSO

Spectrum 6.



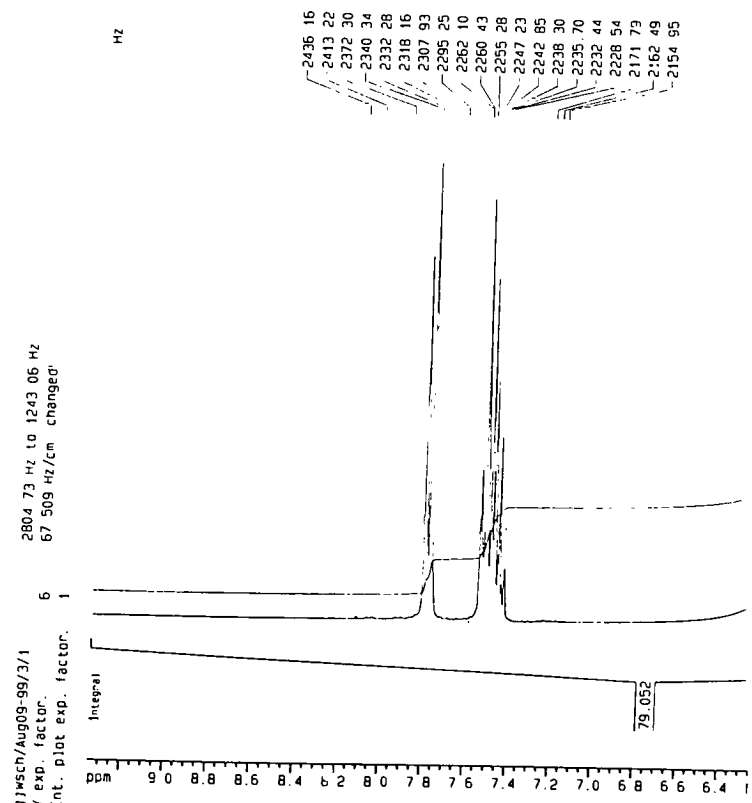
N METHYL ANILINE + AQUEOUS HBr + DMSO

Spectrum 7.



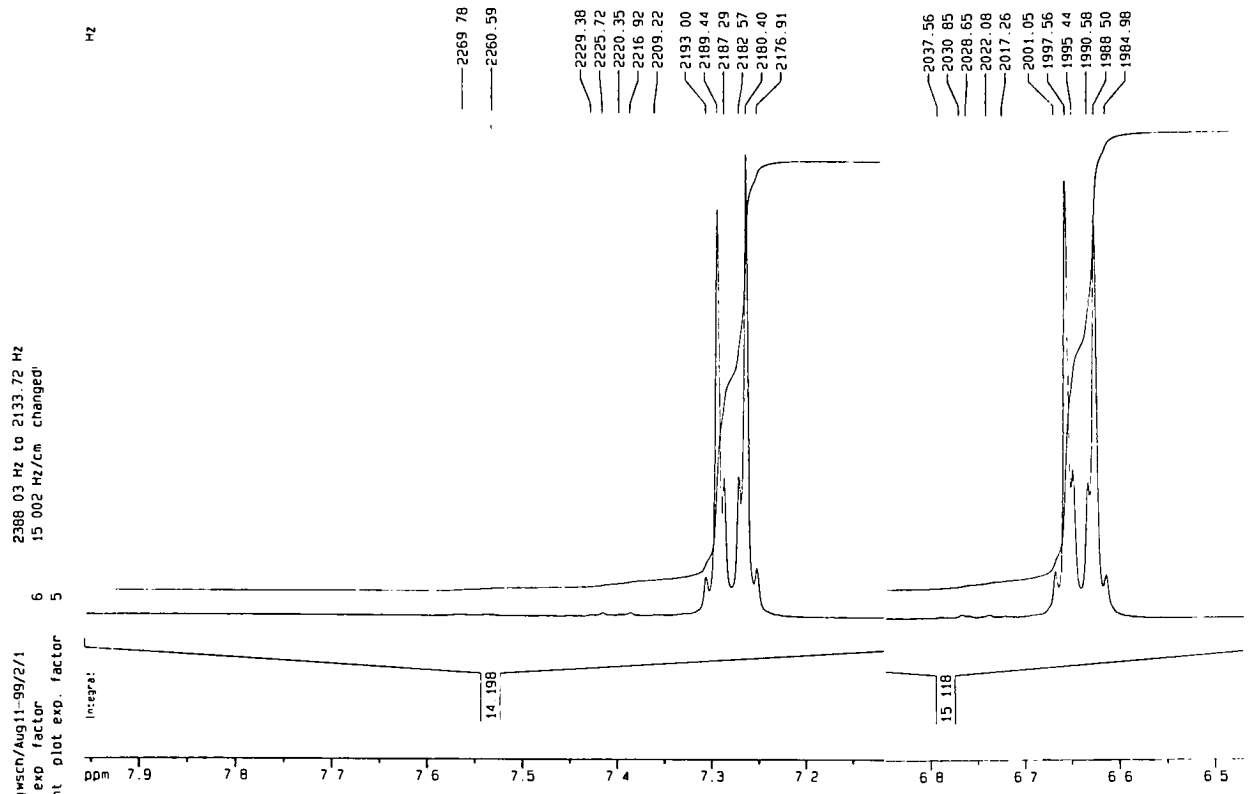
N,N DIMETHYL ANILINE (STANDARD) + DMSO

Spectrum 8.



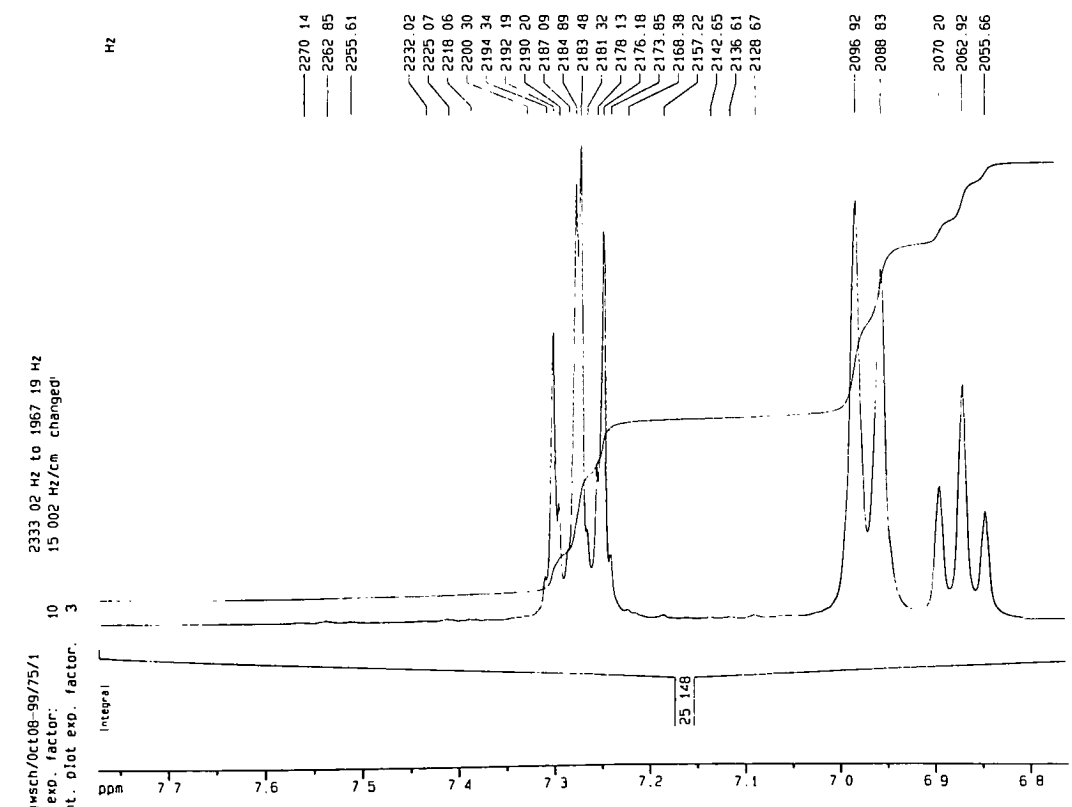
N,N DIMETHYL ANILINE + AQUEOUS HCl + DMSO

Spectrum 9.



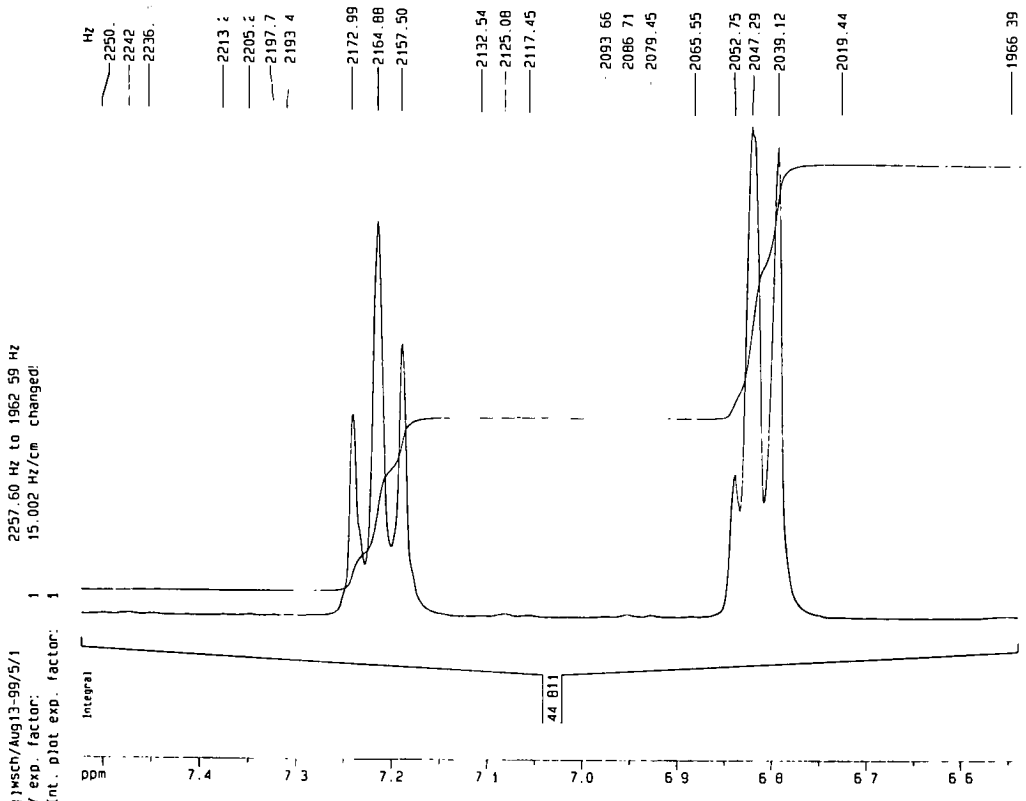
N,N DIMETHYL ANILINE + AQUEOUS HBr + DMSO

Spectrum 10.



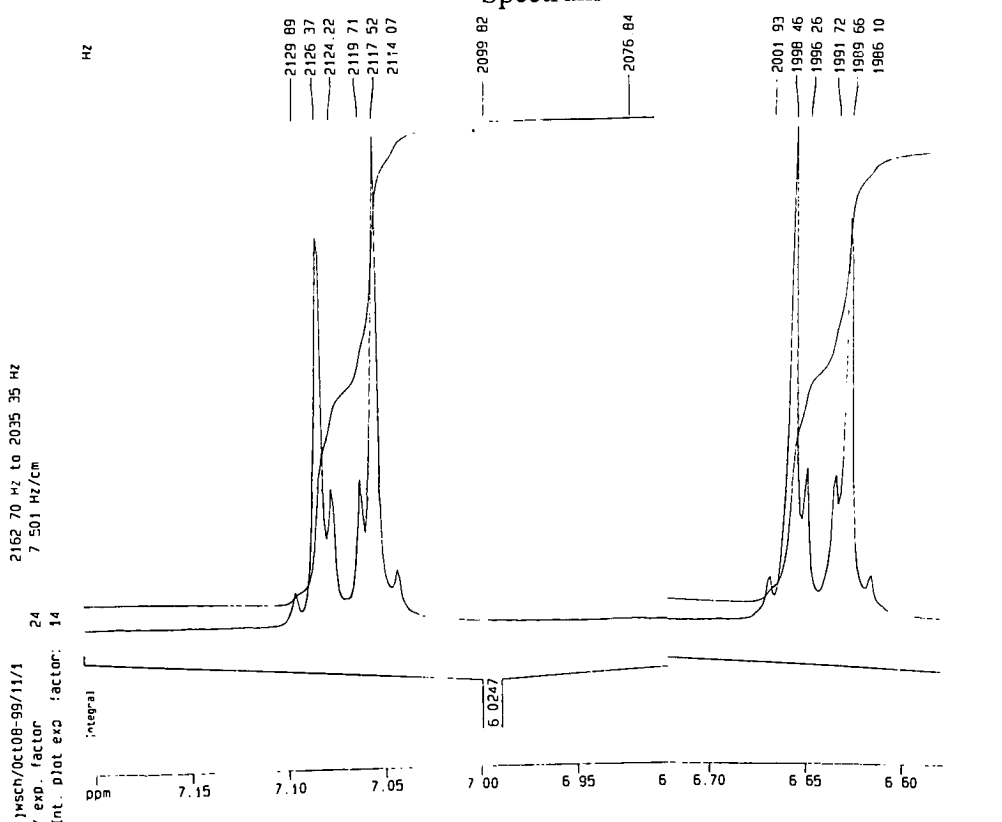
N,N DIMETHYL ANILINE +AQUEOUS HI + DMSO

Spectrum 11.



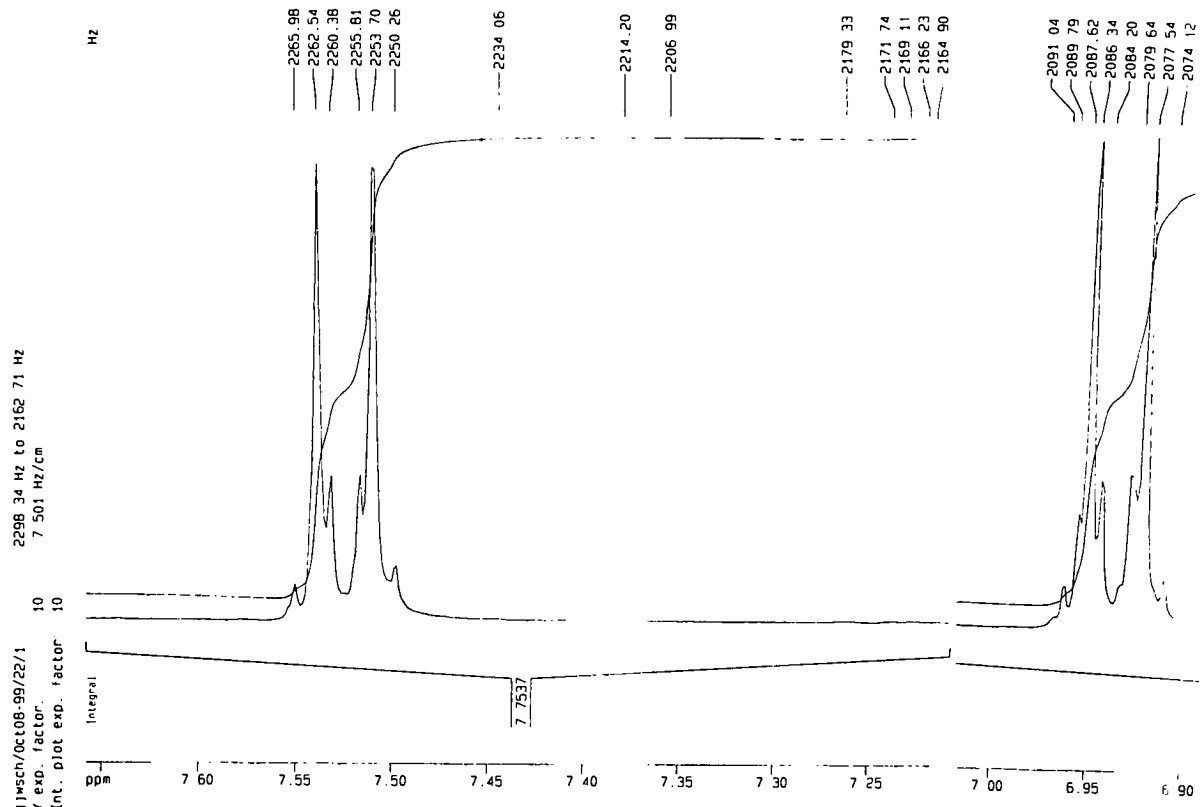
PHENOL (STANDARD) + DMSO

Spectrum 12.



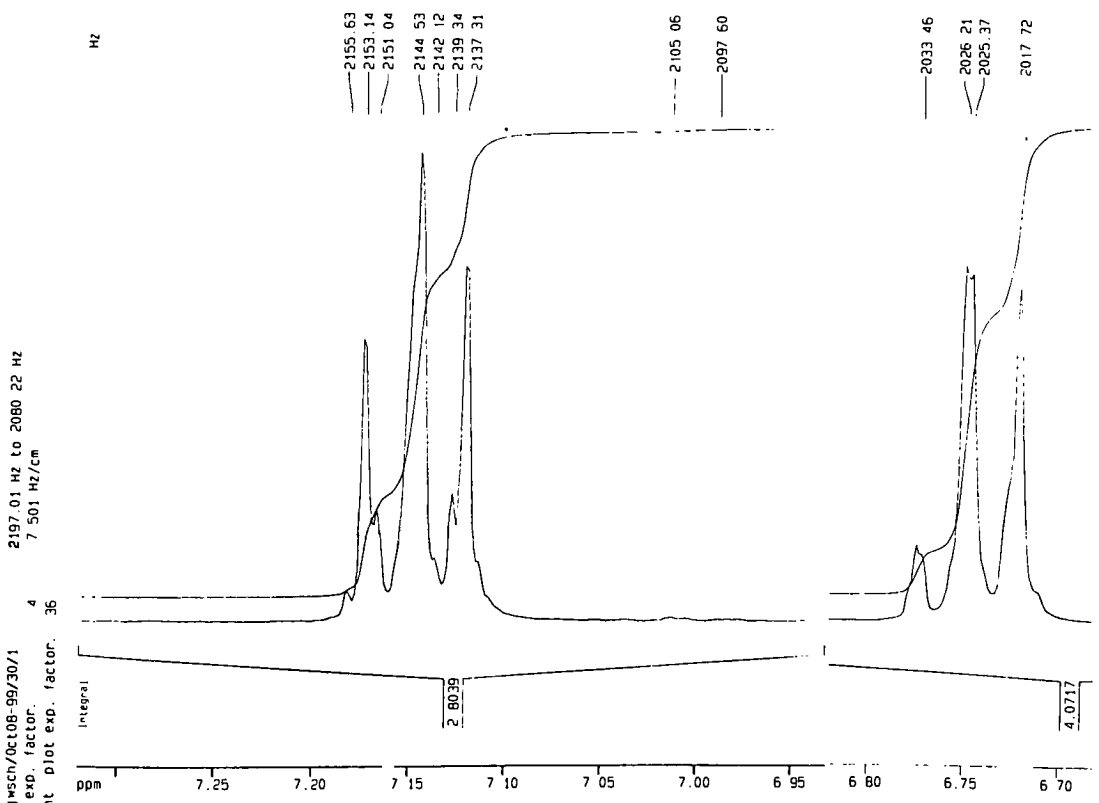
PHENOL + AQUEOUS HCl + DMSO

Spectrum 13.



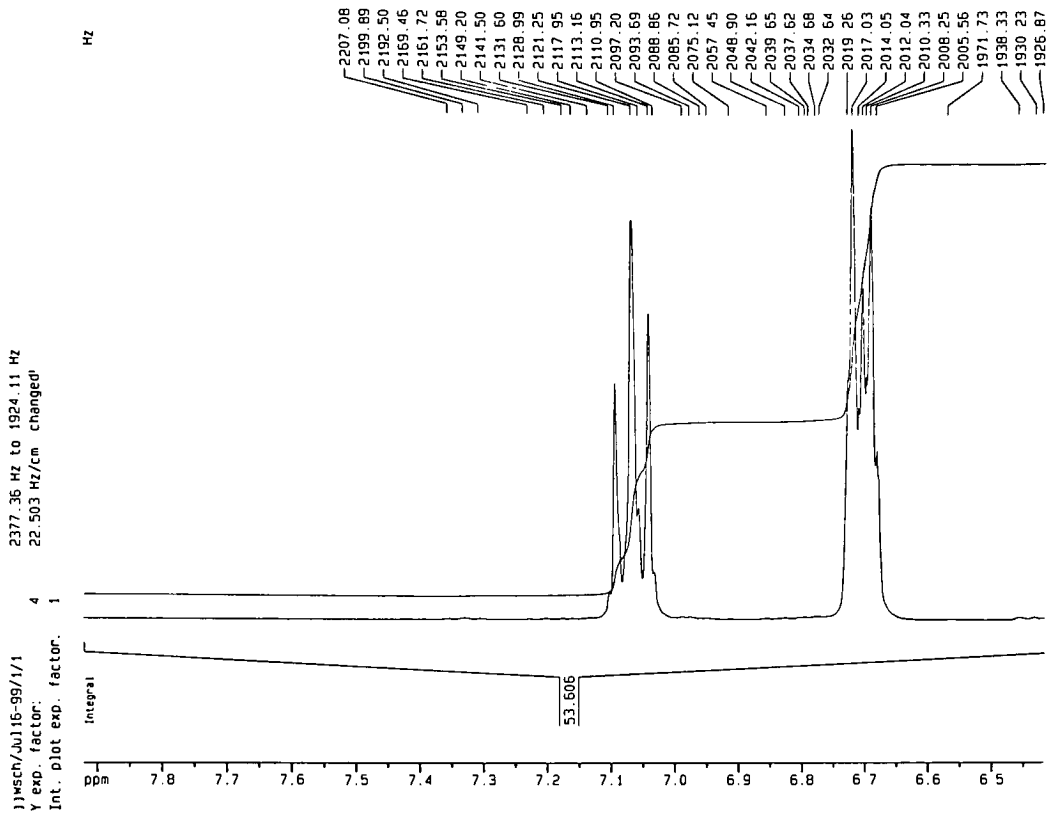
PHENOL + AQUEOUS HBr + DMSO

Spectrum 14.



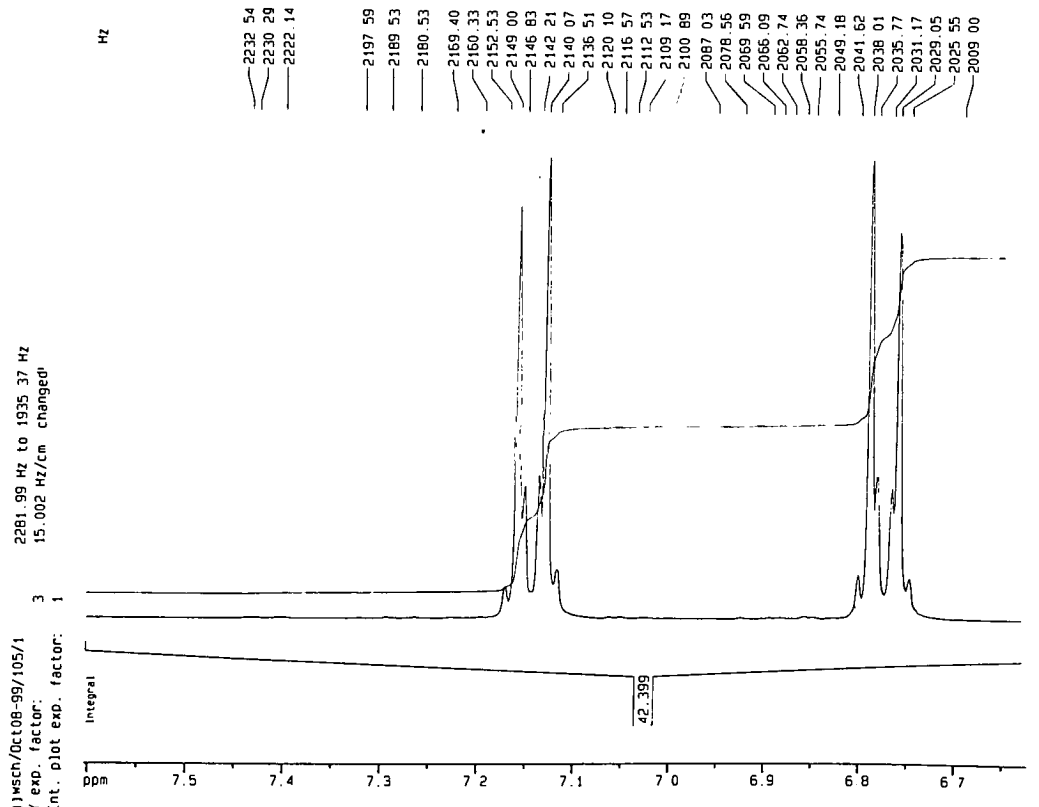
PHENOL + AQUEOUS HI + DMSO

Spectrum 15.



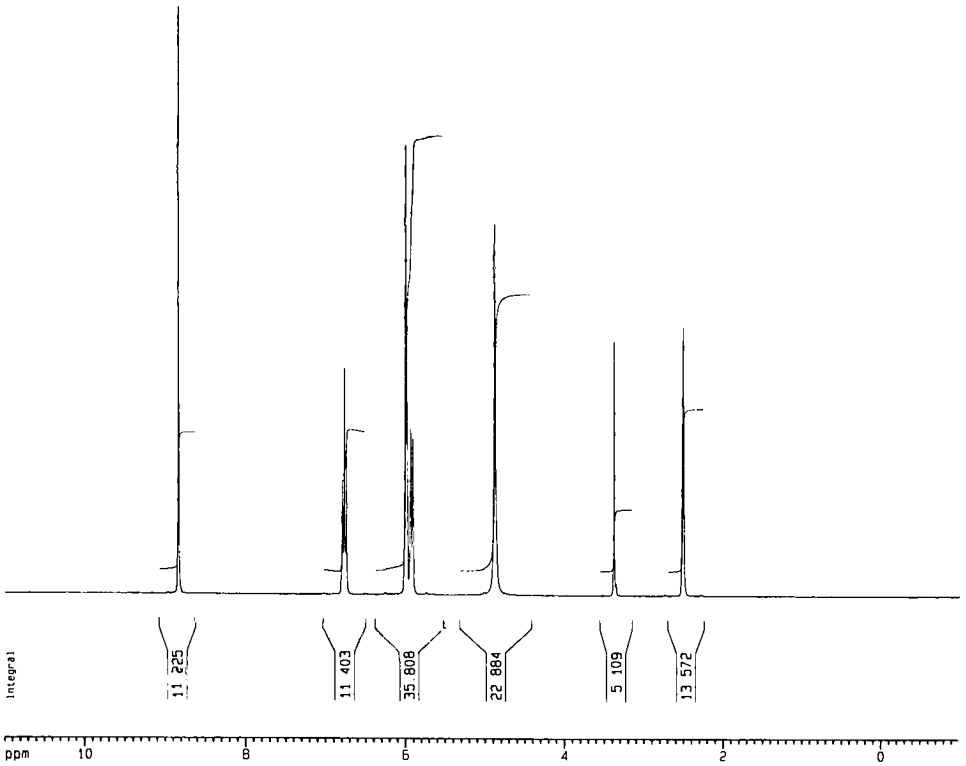
ANISOLE (STANDARD) + DMSO

Spectrum 16.



ANISOLE + GASEOUS HCL + DMSO

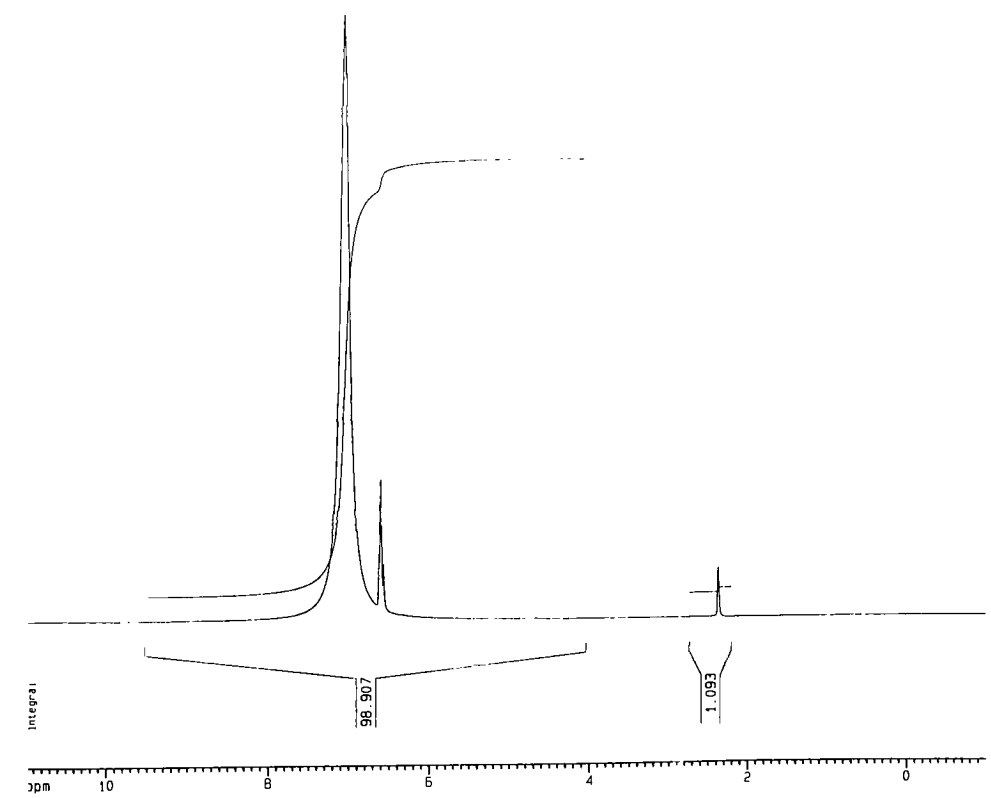
Spectrum 17.




```
Current Data Parameters
NAME                  Jul24-00
EXPNO                        1
PROCNO                       1

F2 - Acquisition Parameters
Date_                   500000
Time                     16 05
INSTRUM                  spect
PROBHD     5 mm ONP  1H
PULPROG                  zg30
TD                       32768
SOLVENT                   DMSO
NS                           16
DS                            2
SWH                 6172 839 Hz
FIDRES              0 188380 Hz
AQ                  2 6542580 sec
RG                          256
DW                   81 000 usec
DE                    6 00 usec
TE                    300 0 K
D1                 1 00000000 sec
P1                    9 00 usec
DE                    6 00 usec
SFO1               300 1318534 MHz
NUC1                        1H
PL1                   -5 00 dB

F2 - Processing parameters
SI                       16384
SF                 300 1300000 MHz
WDW                         EM
SSB                          0
LB                    0 30 Hz
GB                           0
PC                    1 00

1D NMR plot parameters
CX                    20 00 cm
F1P                  11 000 ppm
F1                  3301 43 Hz
F2P                  -1 000 ppm
F2                  -300 13 Hz
PPMCM                0 60000 ppm/cm
HZCM                180 07800 Hz/cm
```

O-PHENYL PHENOL (STANDARD) + DMSO

Spectrum 18.

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Current Data Parameters
NAME                  Jul24-00
EXPNO                        2
PROCNO                       1

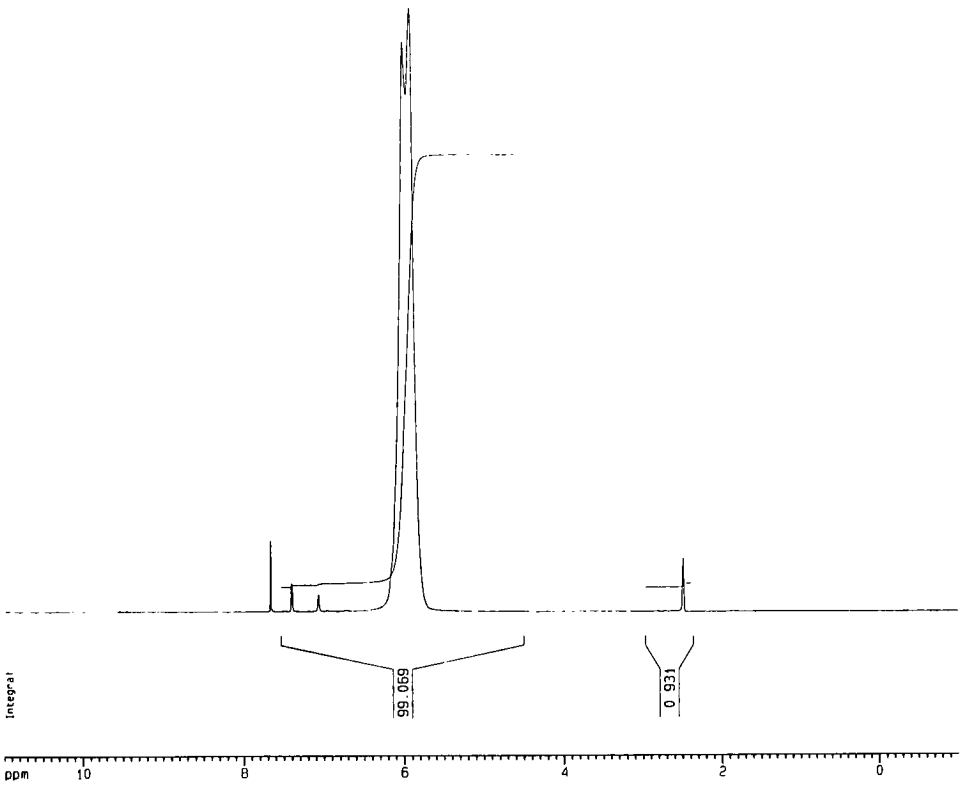
F2 - Acquisition Parameters
Date_                   500000
Time                     16 18
INSTRUM                  sdect
PROBHD     5 mm ONP  1H
PULPROG                  zg30
TD                       32768
SOLVENT                   DMSO
NS                           16
DS                            2
SWH                 6172 839 Hz
FIDRES              0 188380 Hz
AQ                  2 6542580 sec
RG                         28.5
DW                   81 000 usec
DE                    6 00 usec
TE                    300 0 K
D1                 1 00000000 sec
P1                    9 00 usec
DE                    6 00 usec
SFO1              300.1318534 MHz
NUC1                        1H
PL1                   -5 00 dB

F2 - Processing parameters
SI                       16384
SF                 300 1300393 MHz
WDW                         EM
SSB                          0
LB                    0 30 Hz
GB                           0
PC                    1 00

1D NMR plot parameters
CX                    20 00 cm
F1P                  11 000 ppm
F1                  3301 43 Hz
F2P                   -1 000 ppm
F2                  -300 13 Hz
PPMCM                0 60000 ppm/cm
HZCM                180 07802 Hz/cm
```

O-PHENYL PHENOL + AQUEOUS HCl + DMSO

Spectrum 19.




```
Current Data Parameters
NAME                 Jun19-00
EXPNO                       7
PROCNO                      1

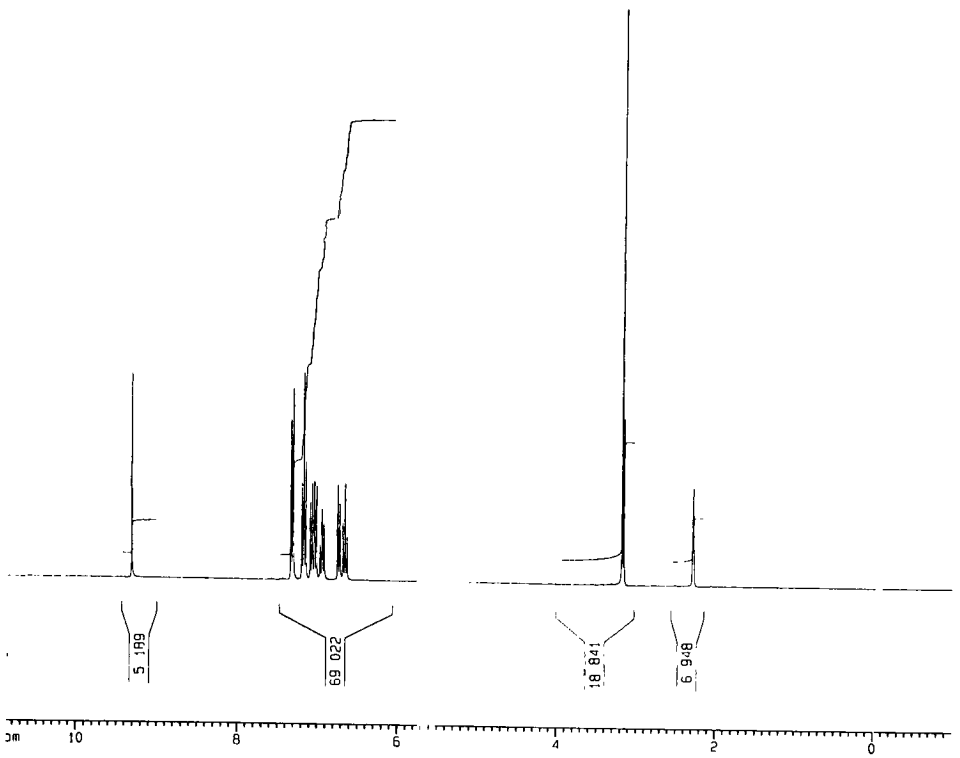
F2 - Acquisition Parameters
Date_                  500000
Time                    17 18
INSTRUM                 spect
PROBHD     5 mm QNP  1H
PULPROG                 zg30
TD                     32768
SOLVENT                 DMSO
NS                        16
DS                         2
SWH                6172 839 Hz
FIDRES             0 188380 Hz
AQ                 2 6542580 sec
RG                      25.4
DW                   81 000 usec
DE                    6.00 usec
TE                    300 0 K
D1                 1 00000000 sec
P1                    9 00 usec
DE                    6 00 usec
SFO1              300 1318534 MHz
NUC1                      1H
PL1                   -5 00 dB

F2 - Processing parameters
SI                      16384
SF                300 1300000 MHz
WDW                        EM
SSB                         0
LB                      0 30 Hz
GB                          0
PC                       1.00

1D NMR plot parameters
CX                     20.00 cm
F1P                    11 000 ppm
F1                   3301 43 Hz
F2P                    -1 000 ppm
F2                   -300 13 Hz
PPMCM                0 60000 ppm/cm
HZCM                180 07800 Hz/cm
```

O-PHENYL PHENOL + AQUEOUS HBr + DMSO

Spectrum 20.




```
Current Data Parameters
NAME            Jun19-00
EXPNO                  5
PROCNO                 1

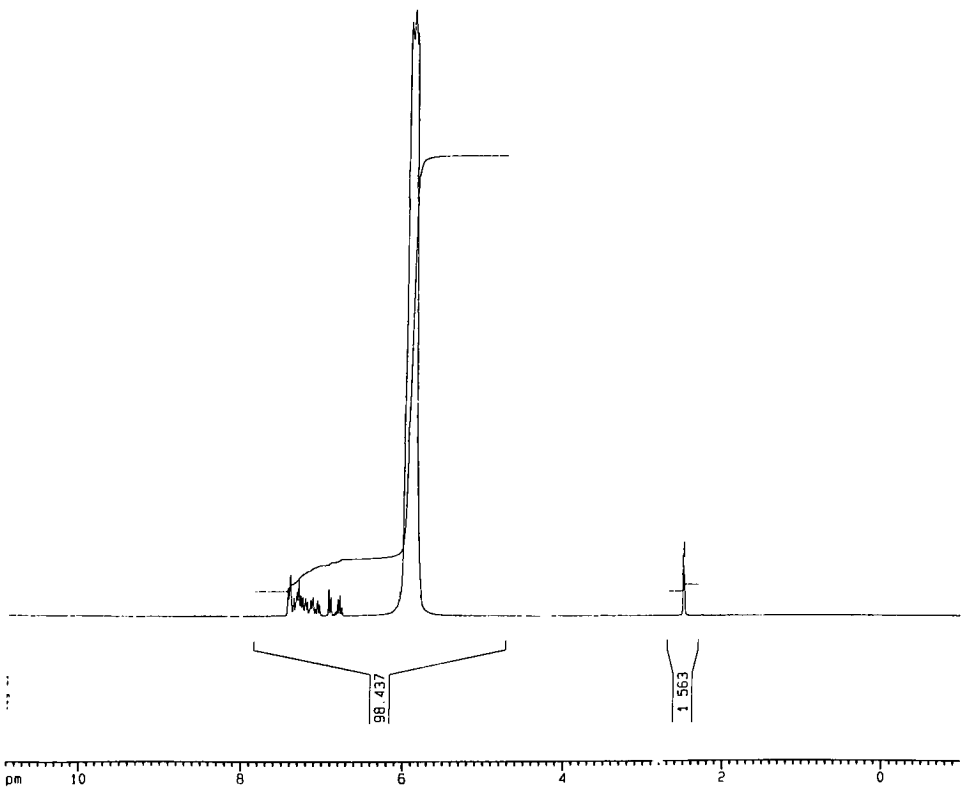
F2 - Acquisition Parameters
Date_             500000
Time               16 40
INSTRUM            spect
PROBHD     5 mm ONP  1H
PULPROG             zg30
TD                 32768
SOLVENT             DMSO
NS                    16
DS                     2
SWH           6172 839 Hz
FIDRES        0 188380 Hz
AQ            2.6542580 sec
RG                   181
DW               81 000 usec
DE                6 00 usec
TE               300 0 K
D1            1 00000000 sec
P1                9 00 usec
DE                6 00 usec
SFO1         300 1318534 MHz
NUC1                 1H
PL1               -5 00 dB

F2 - Processing parameters
SI                16384
SF          300 1300638 MHz
WDW                  EM
SSB                   0
LB                 0 30 Hz
GB                    0
PC                 1 00

10 NMR plot parameters
CX                20 00 cm
F1P               11 000 ppm
F1                3301 43 Hz
F2P               -1 000 ppm
F2                -300 13 Hz
PPMCM          0 60000 ppm/cm
HZCM          180 07803 Hz/cm
```

3-AMINO PHENOL (STANDARD) + DMSO

Spectrum 21.

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Current Data Parameters
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EXPNO                  4
PROCNO                 1

F2 - Acquisition Parameters
Date_             500000
Time               16 29
INSTRUM            spect
PROBHD     5 mm ONP  1H
PULPROG             zg30
TD                 32768
SOLVENT             DMSO
NS                    16
DS                     2
SWH           6172 839 Hz
FIDRES        0 188380 Hz
AQ            2 6542580 sec
RG                  35 9
DW               81 000 usec
DE                6 00 usec
TE               300 0 K
D1            1 00000000 sec
P1                9 00 usec
DE                6 00 usec
SFO1         300 1318534 MHz
NUC1                 1H
PL1               -5 00 dB

F2 - Processing parameters
SI                16384
SF          300 1300000 MHz
WDW                  EM
SSB                   0
LB                 0 30 Hz
GB                    0
PC                 1 00

10 NMR plot parameters
CX                20 00 cm
F1P               11 000 ppm
F1                3301 43 Hz
F2P               -1 000 ppm
F2                -300 13 Hz
PPMCM          0 60000 ppm/cm
HZCM          180 07800 Hz/cm
```
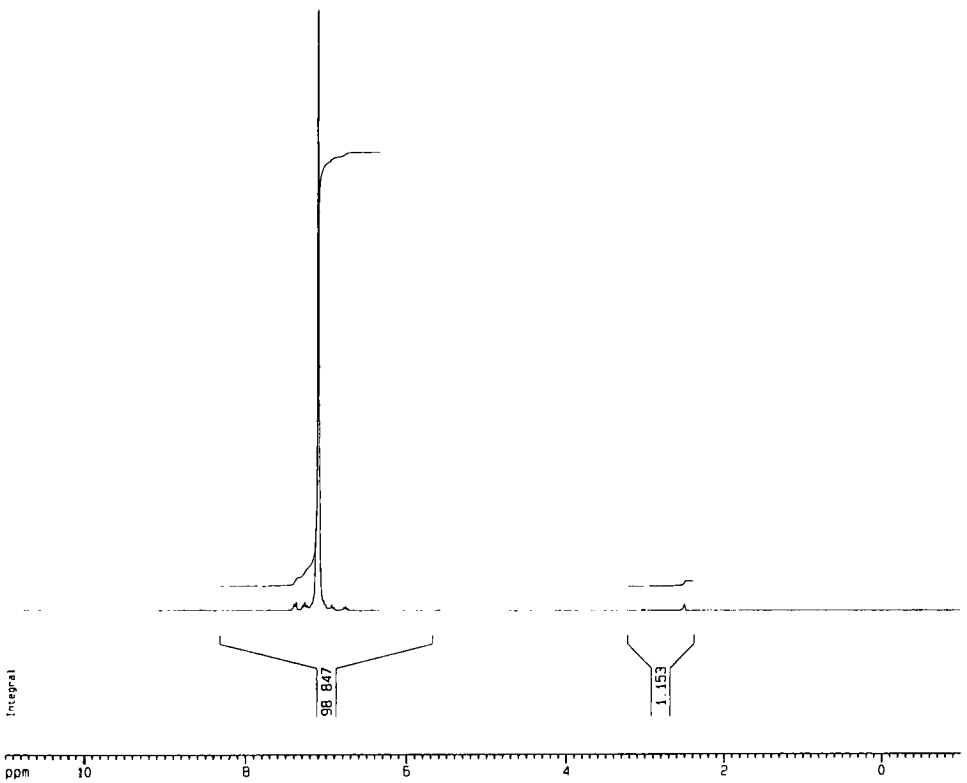

3-AMINO PHENOL + AQUEOUS HCl + DMSO

Spectrum 22.




```
Current Data Parameters
NAME                  Jun19-00
EXPNO                        6
PROCNO                       1

F2 - Acquisition Parameters
Date_                   500000
Time                     16 51
INSTRUM                  spect
PROBHD     5 mm ONP  1H
PULPROG                  zg30
TD                      32768
SOLVENT                  DMSO
NS                         16
DS                          2
SWH                6172 839 Hz
FIDRES             0 188380 Hz
AQ                 2 6542580 sec
RG                      28 5
DW                    81 000 usec
DE                    6 00 usec
TE                    300 0 K
D1                1 00000000 sec
P1                    9 00 usec
DE                    6 00 usec
SFO1             300 1318534 MHz
NUC1                      1H
PL1                   -5 00 dB

F2 - Processing parameters
SI                       16384
SF               300 1300000 MHz
WDW                        EM
SSB                         0
LB                     0 30 Hz
GB                          0
PC                     1 00

1D NMR plot parameters
CX                    20 00 cm
F1P                  11 000 ppm
F1                  3301 43 Hz
F2P                  -1 000 ppm
F2                  -300.13 Hz
PPMCM               0 60000 ppm/cm
HZCM               180 07800 Hz/cm
```

3-AMINO PHENOL + AQUEOUS HBr + DMSO

APPENDIX B

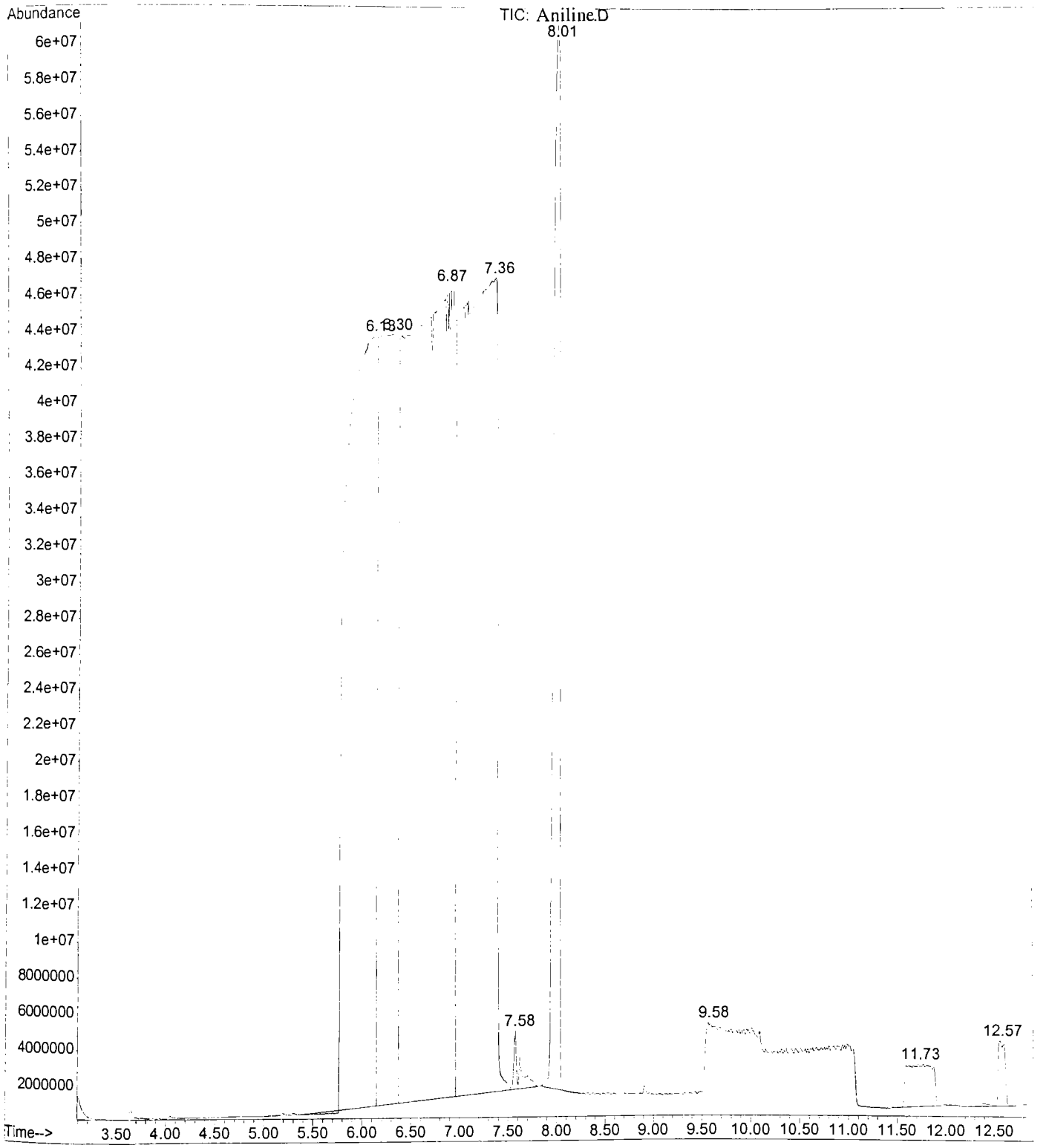
GC Spectrum 1. Area Percent Report

B1

Data File : C:\HPCHEM\1\DATA\ Aniline..D
Acq On : 13 Jul 2000 15:13
Sample :
Misc :

Vial: 1
Operator:
Inst : RIT Chemi
Multiplr: 1.00
Sample Amount: 0.00

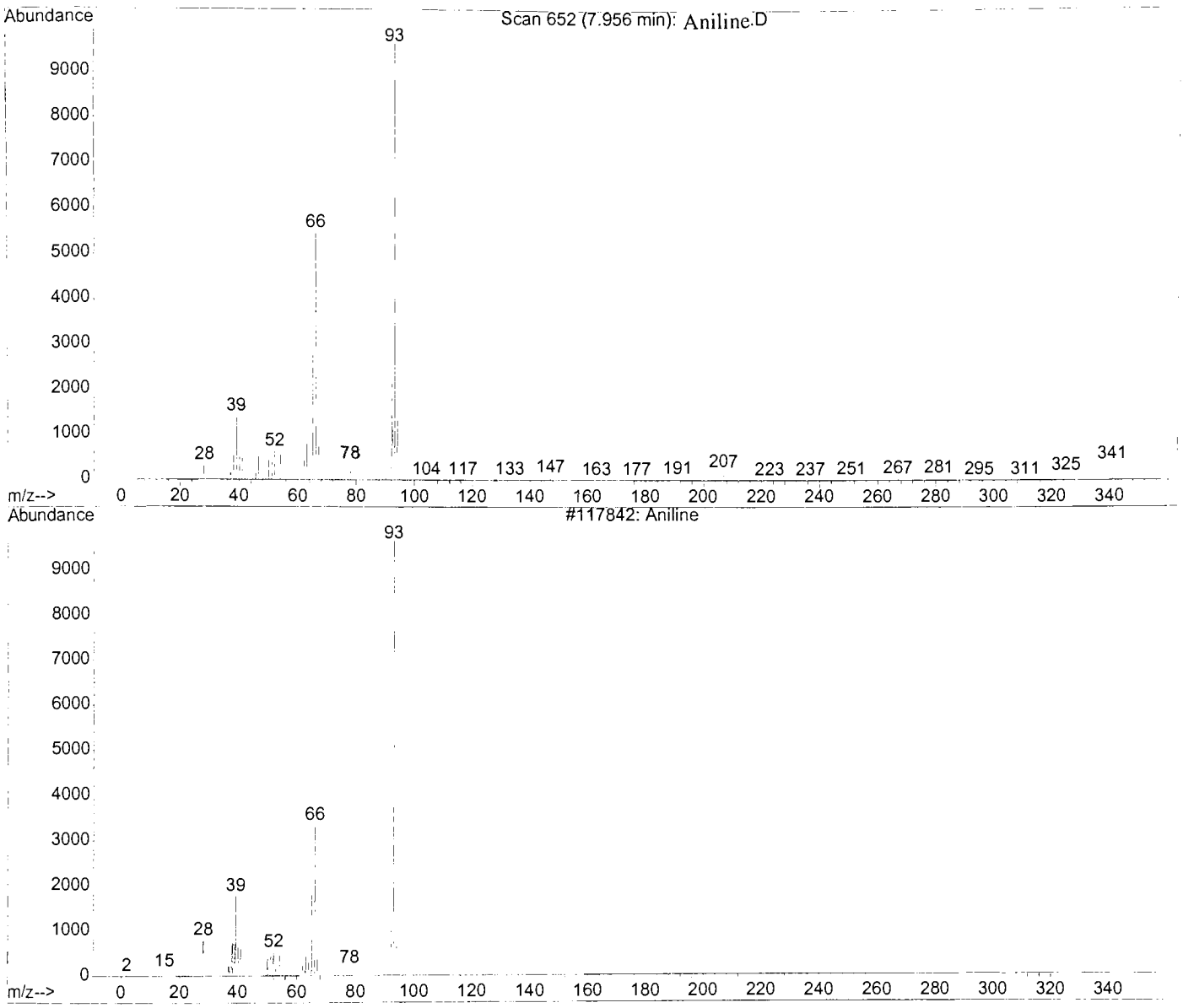
MS Integration Params: autoint1.e
Method : C:\HPCHEM\1\5973\PANKAJ.M (Chemstation Integrator)
Title :



ANIHBR.D PANKAJ.M Thu Jul 13 15:26:49 2000

Library Searched : C:\DATABASE\NIST98.L
Quality : 93
ID : Aniline

MS Spectrum 1.



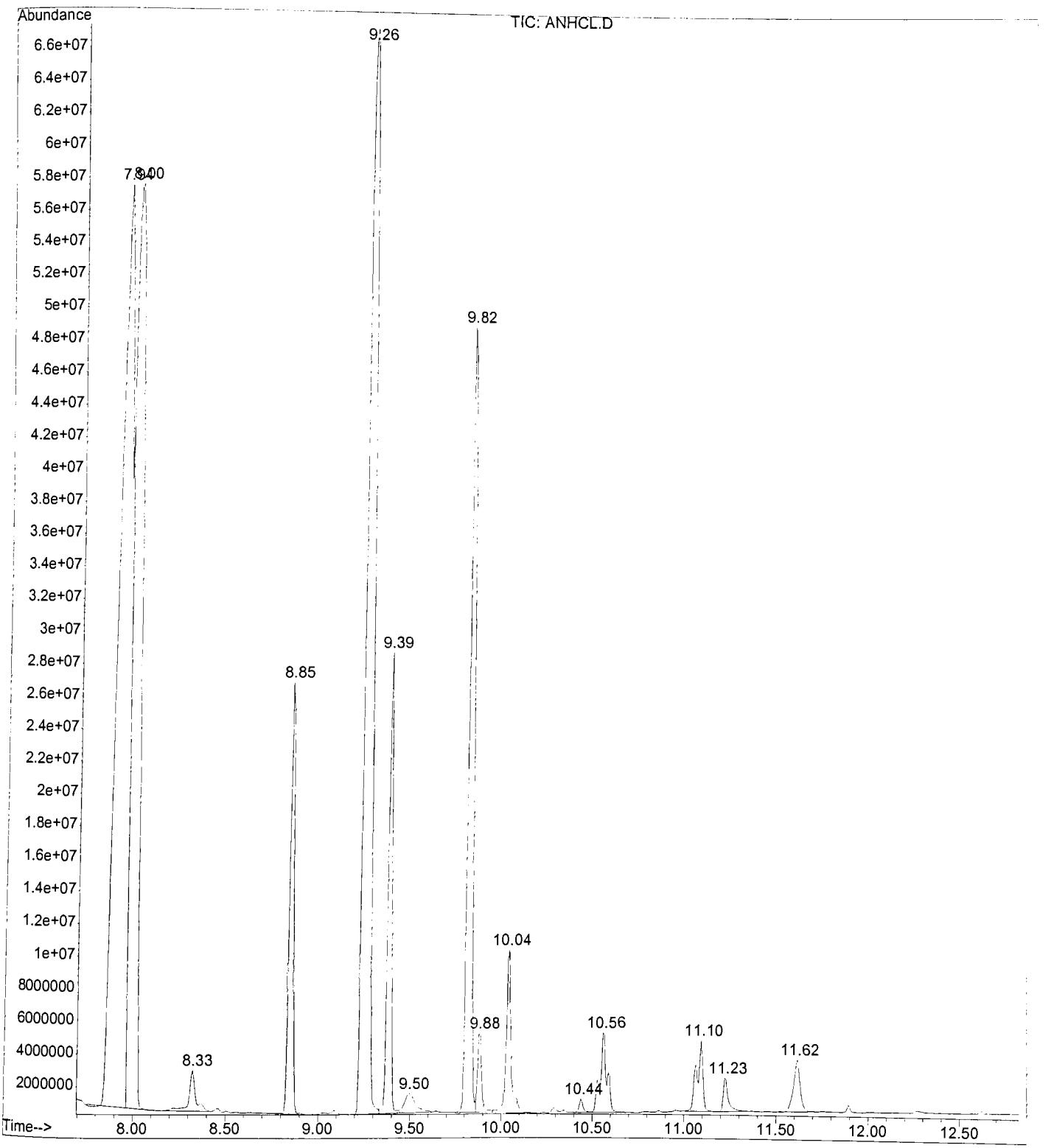
GC Spectrum 2. Area Percent Report

B2

Data File : C:\HPCHEM\1\DATA\ANHCL.D
Acq On : 13 Jul 2000 16:13
Sample :
Misc :

Vial: 1
Operator:
Inst : RIT Chemi
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e
Method : C:\HPCHEM\1\5973\PANKAJ7.M (Chemstation Integrator)
Title :

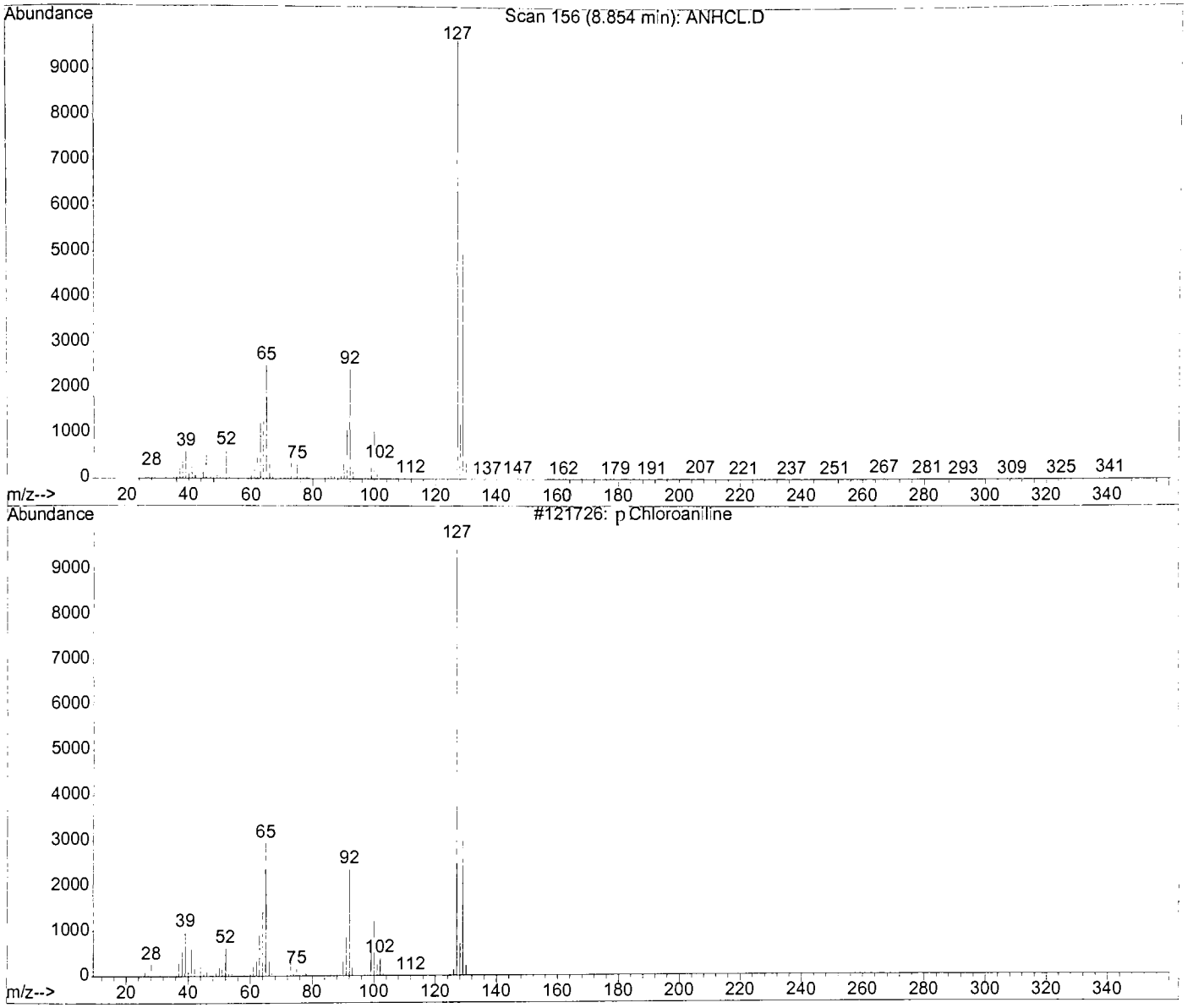


ANHCL.D PANKAJ7.M Thu Jul 13 16:26:43 2000

Library Searched : C:\DATABASE\NIST98.L
Quality : 93
ID : P-Chloroaniline

B2

MS Spectrum 2.

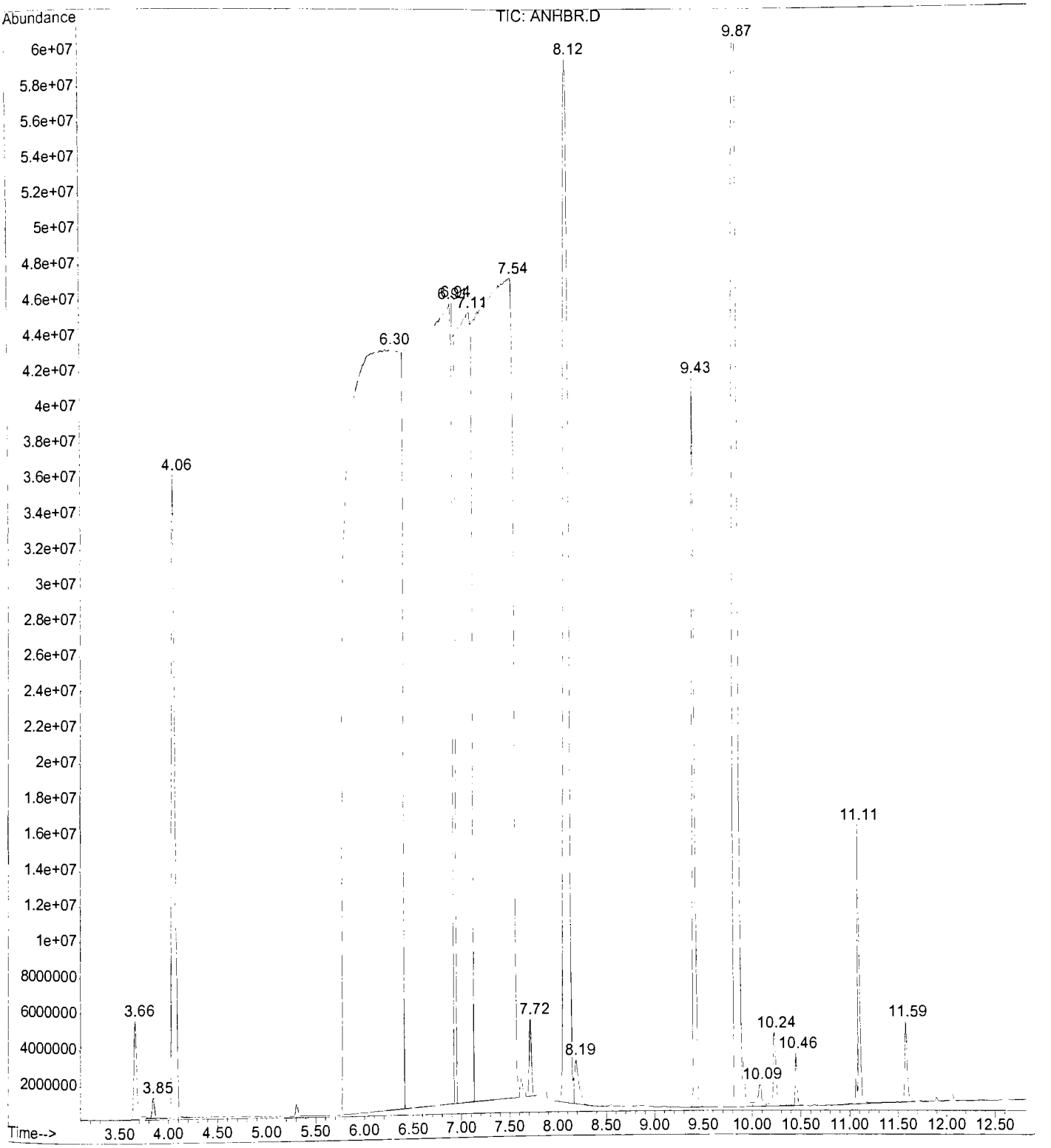


GC Spectrum 3. Area Percent Report

Data File : C:\HPCHEM\1\DATA\ANHBR.D
Acq On : 13 Jul 2000 15:35
Sample :
Misc :

Vial: 1
Operator:
Inst : RIT Chemi
Multiplr: 1.00
Sample Amount: 0.00

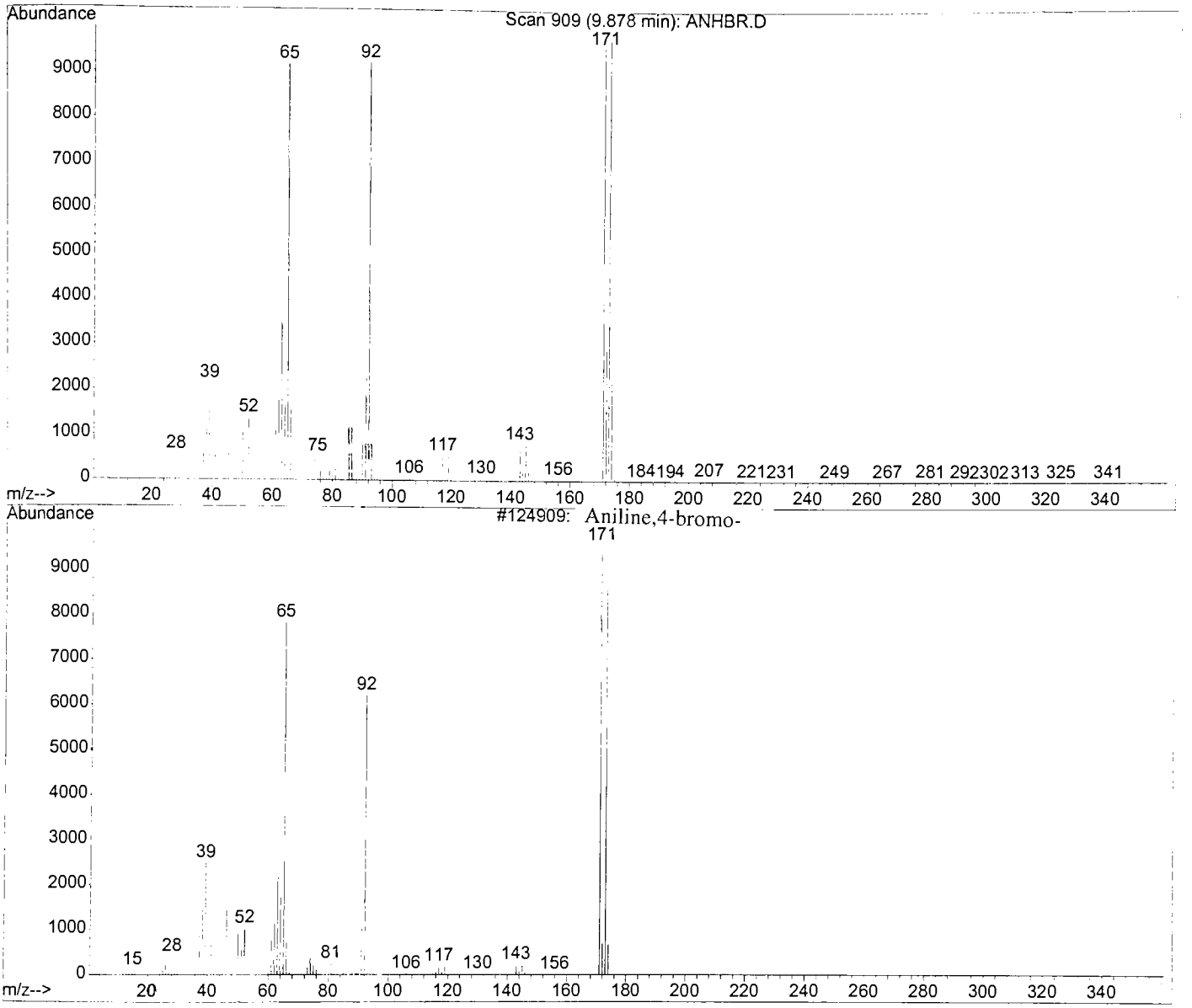
MS Integration Params: autoint1.e
Method : C:\HPCHEM\1\5973\PANKAJ.M (Chemstation Integrator)
Title :



ANHBR.D PANKAJ.M Thu Jul 13 15:48:48 2000

Library Searched : C:\DATABASE\NIST98.L
Quality : 94
ID : Aniline, 4 - bromo-

MS Spectrum 3.

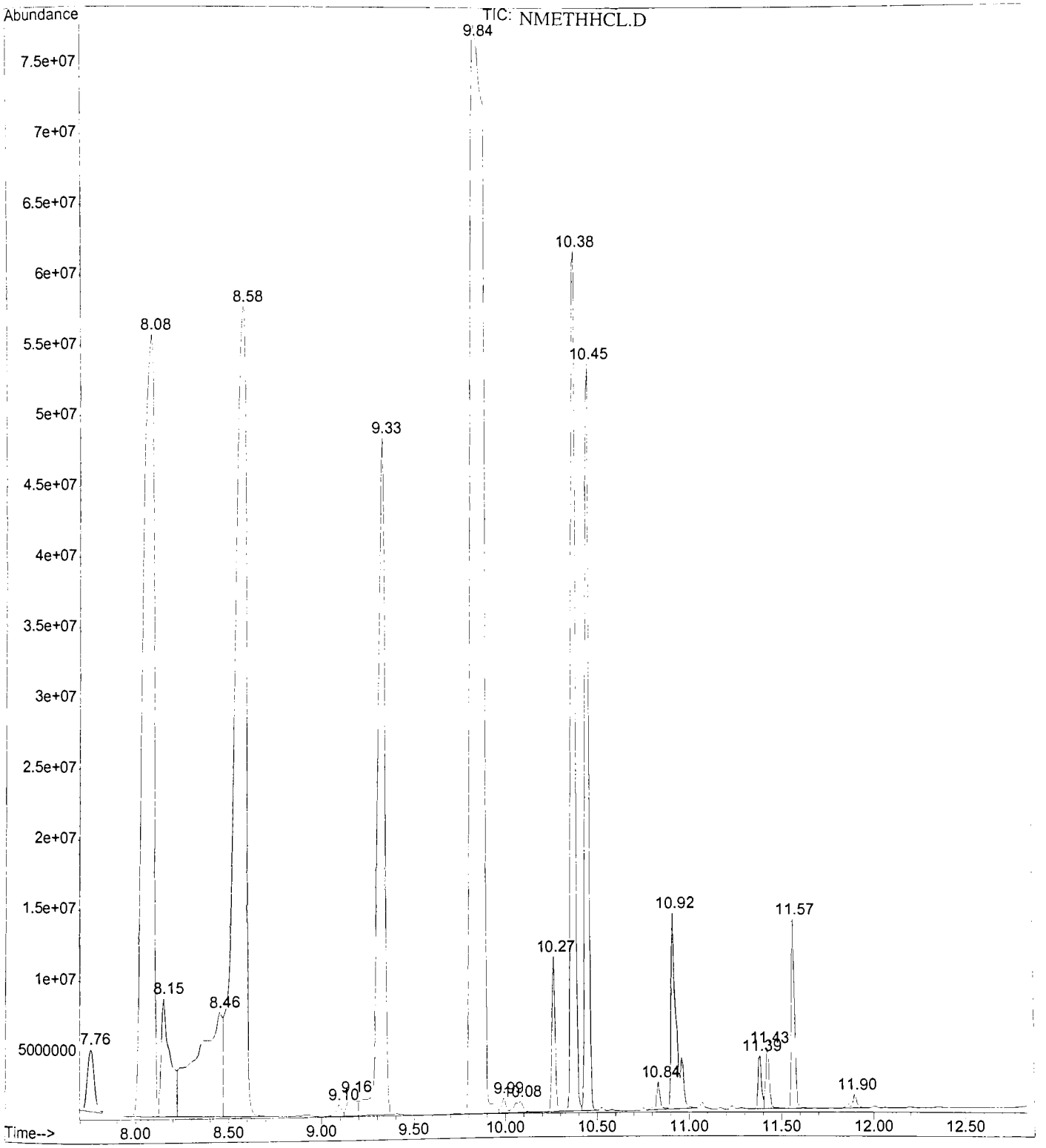


GC Spectrum 4. Area Percent Report

Data File : C:\HPCHEM\1\DATA\NMETHHCl.D
Acq On : 13 Jul 2000 17:54
Sample :
Misc :

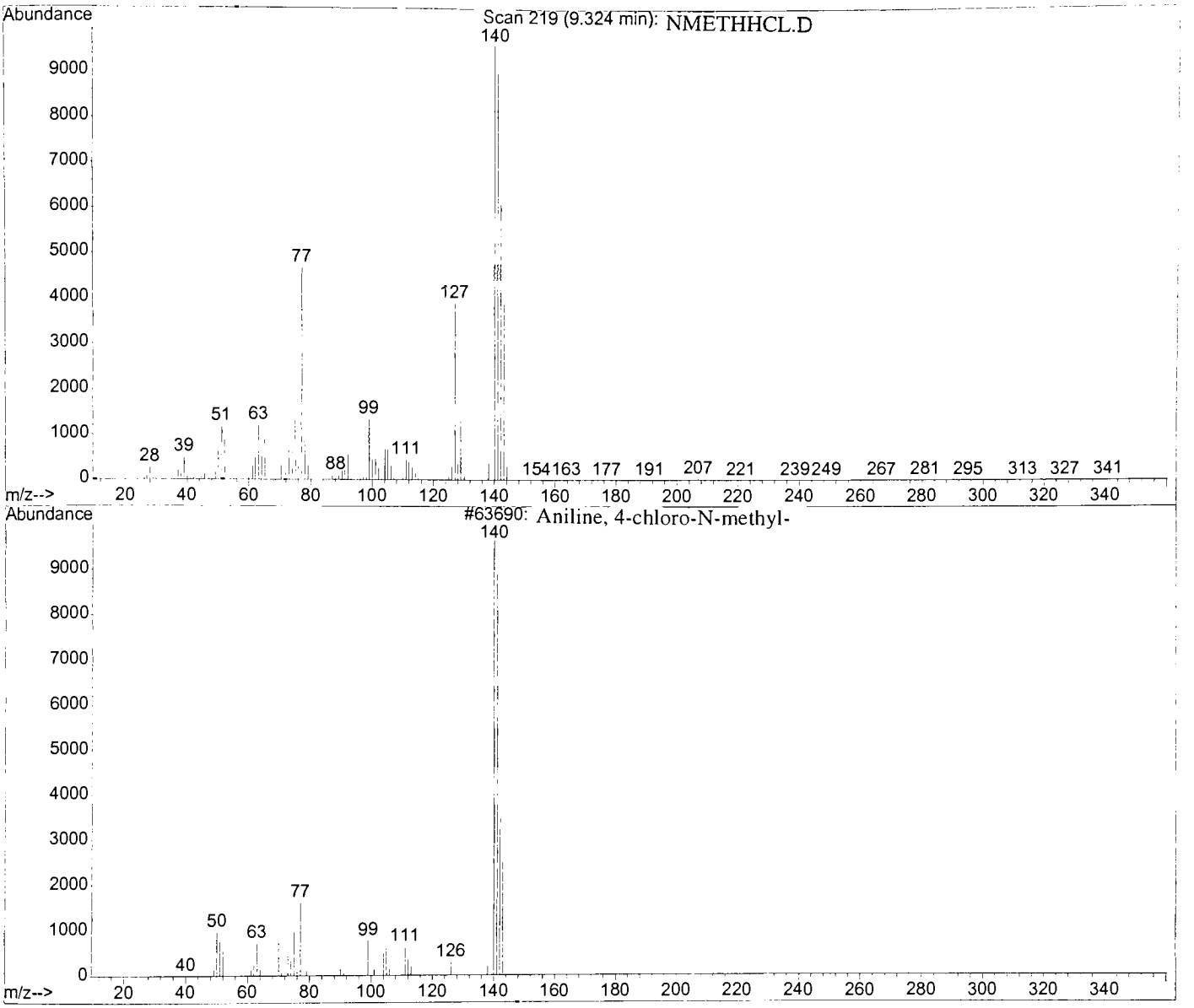
Vial: 1
Operator:
Inst : RIT Chemi
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e
Method : C:\HPCHEM\1\5973\PANKAJ7.M (Chemstation Integrator)
Title :



NMETHHBR.D PANKAJ7.M Thu Jul 13 18:07:28 2000

Library Searched : C:\DATABASE\NIST98.L
Quality : 60
ID : Aniline, 4- chloro- N - methyl-¹ MS Spectrum 4.

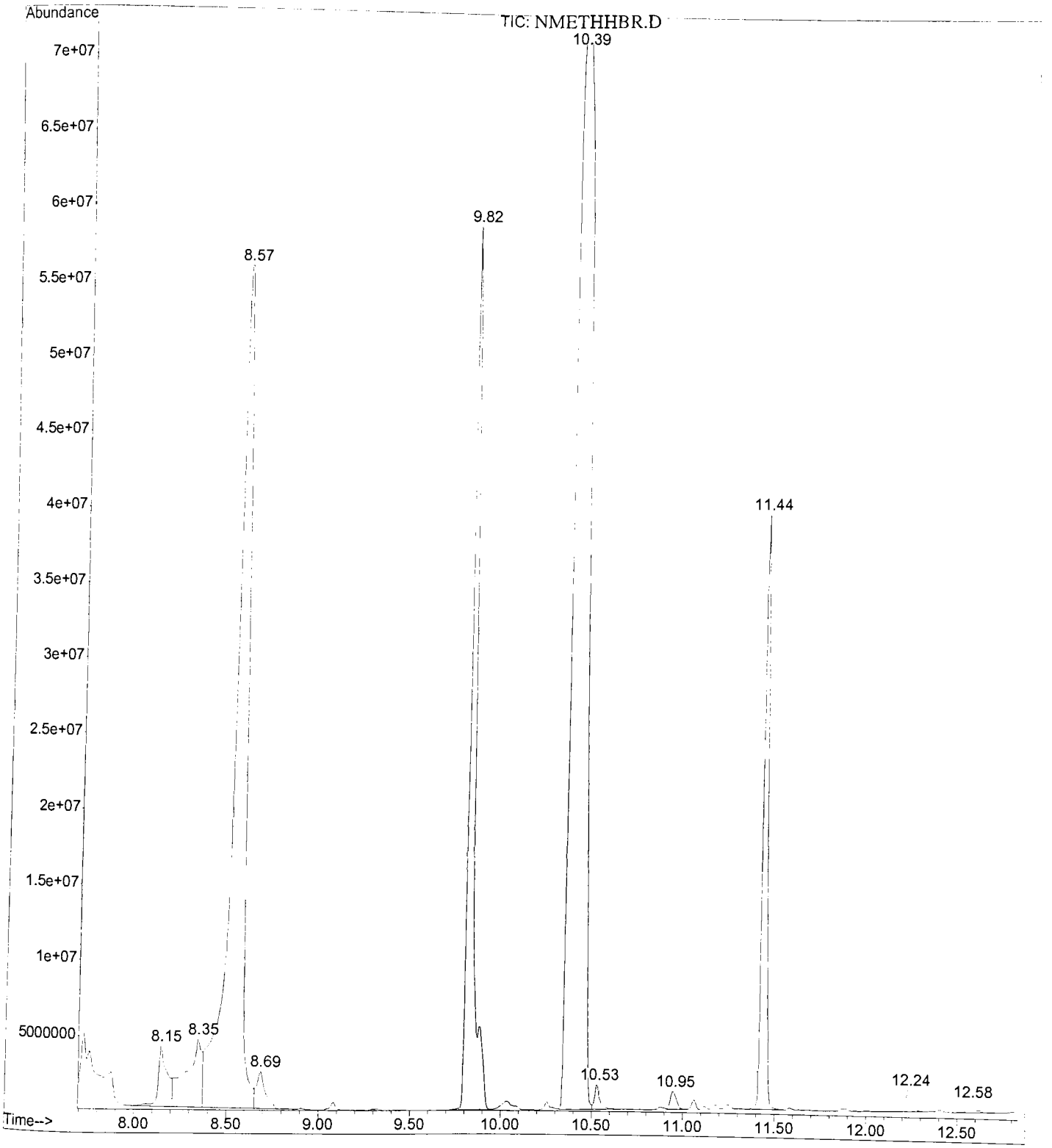


GC Spectrum 5. Area Percent Report

Data File : C:\HPCHEM\1\DATA\NMETHHBR.D
Acq On : 13 Jul 2000 18:40
Sample :
Misc :

Vial: 1
Operator:
Inst : RIT Chemi
Multiplr: 1.00
Sample Amount: 0.00

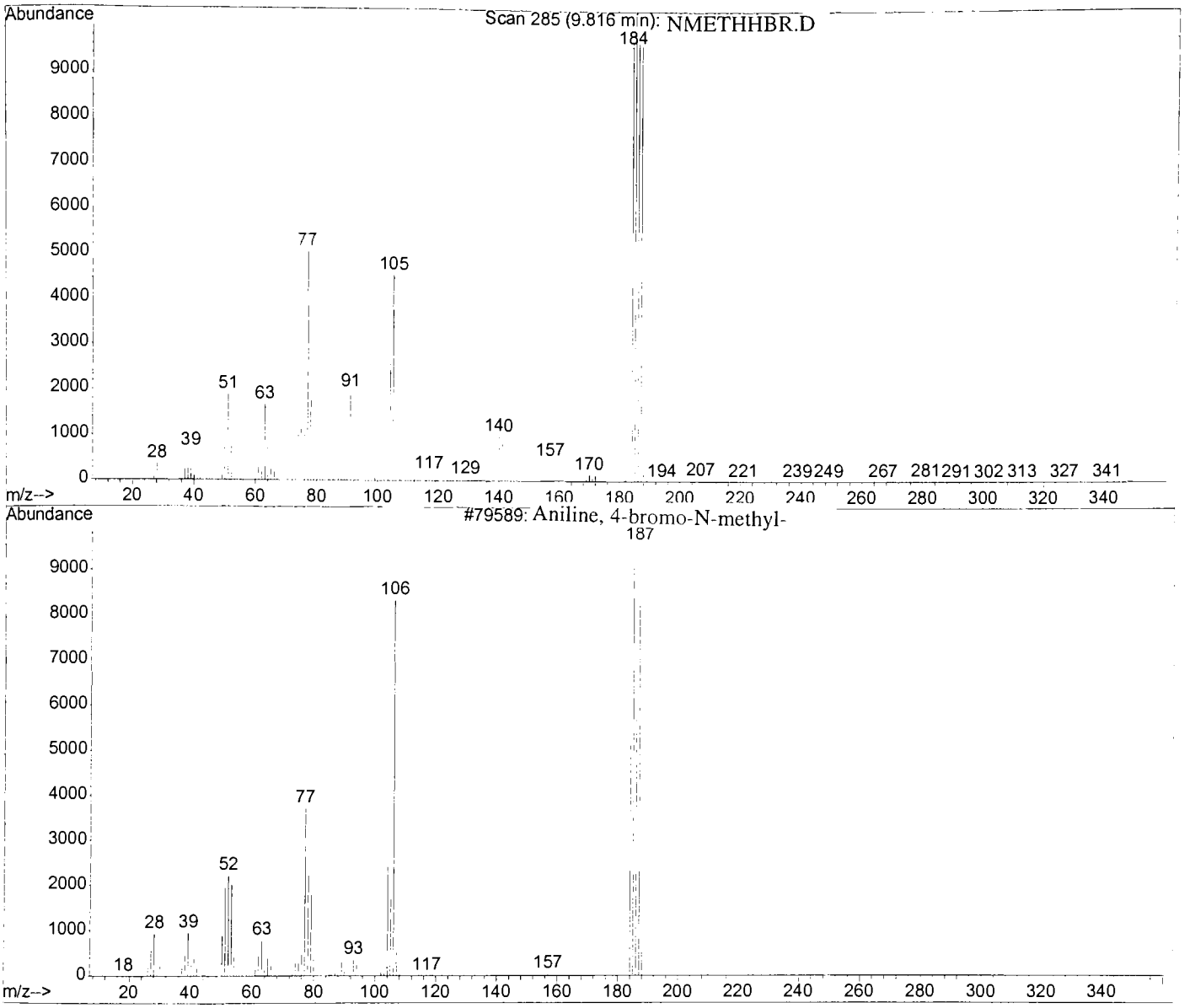
MS Integration Params: autoint1.e
Method : C:\HPCHEM\1\5973\PANKAJ7.M (Chemstation Integrator)
Title :



NMETHHCL.D PANKAJ7.M Thu Jul 13 18:52:55 2000

Library Searched : C:\DATABASE\NIST98.L
Quality : 47
ID : Aniline, 4- bromo- N - methyl-

MS Spectrum 5.

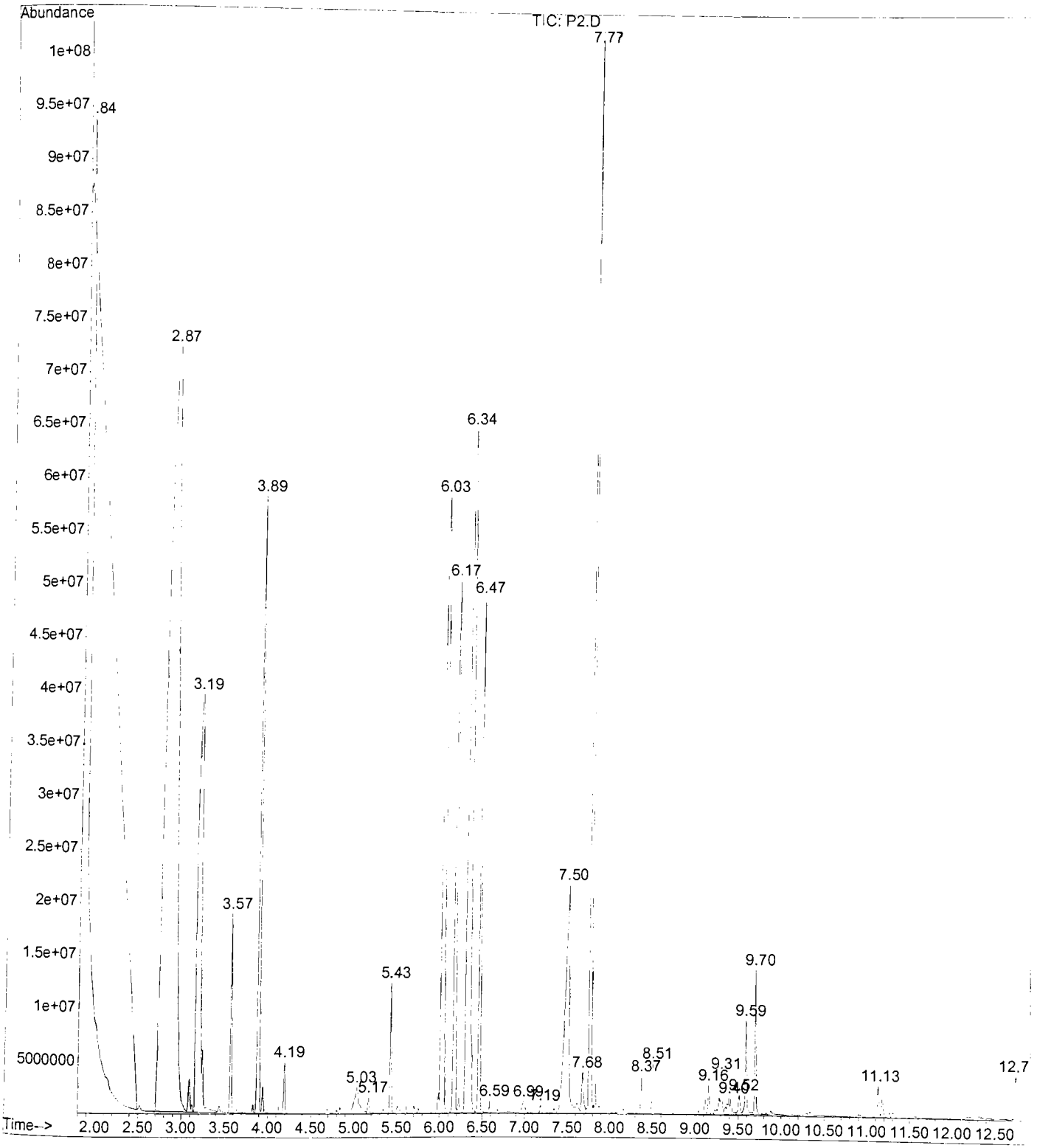


GC Spectrum 6. Area Percent Report

Data File : C:\HPCHEM\1\DATA\P2.D
Acq On : 4 Jan 2000 14:47
Sample : Aniline, 4- bromo- N,N - dimethyl-
Misc :

Vial: 1
Operator: Pankaj
Inst : RIT Chemi
Multiplr: 1.00
Sample Amount: 0.00

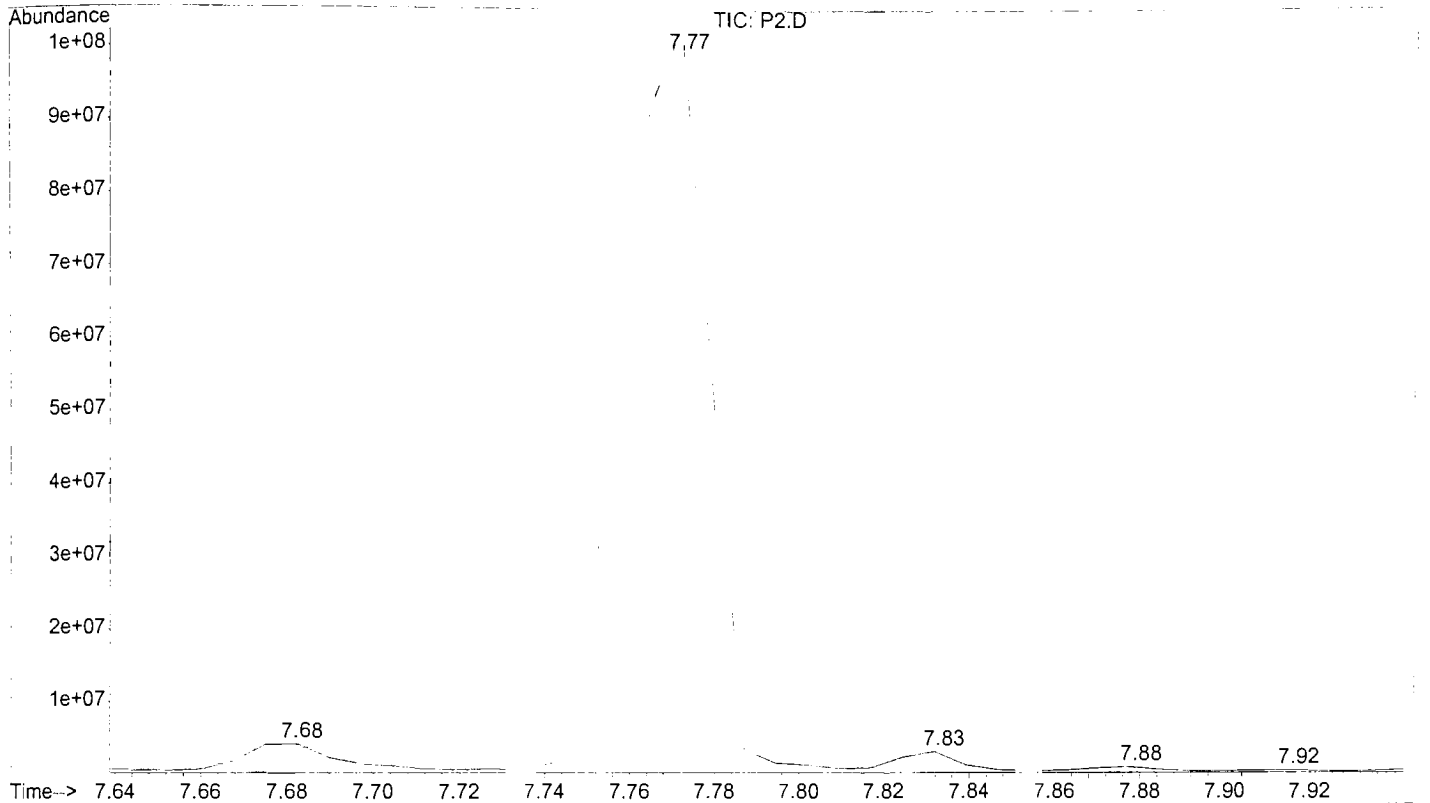
MS Integration Params: autoint1.e
Method : C:\HPCHEM\1\5973\PANKAJ.M (Chemstation Integrator)
Title :

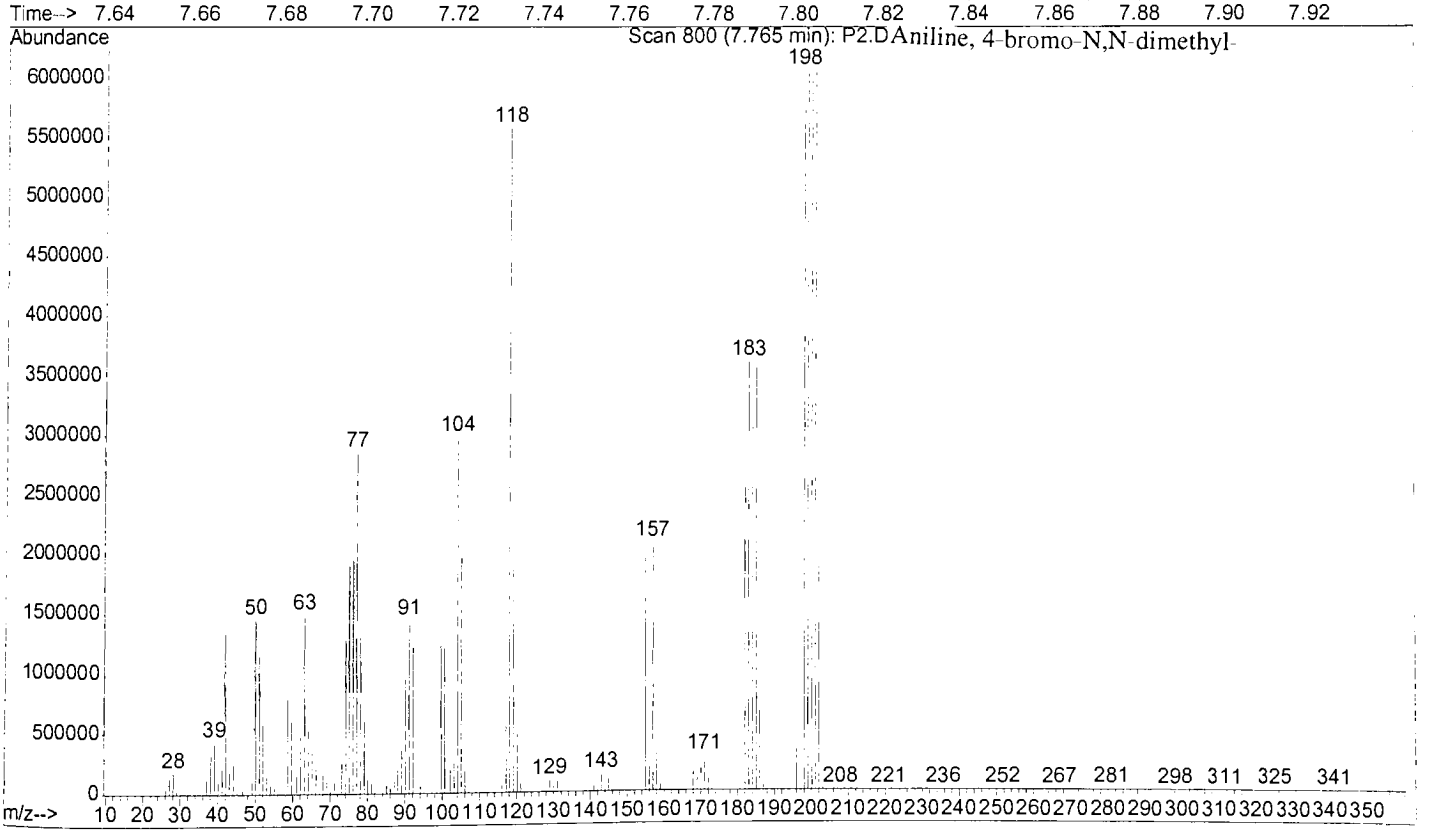


P2.D PANKAJ.M Tue Jan 04 15:00:23 2000

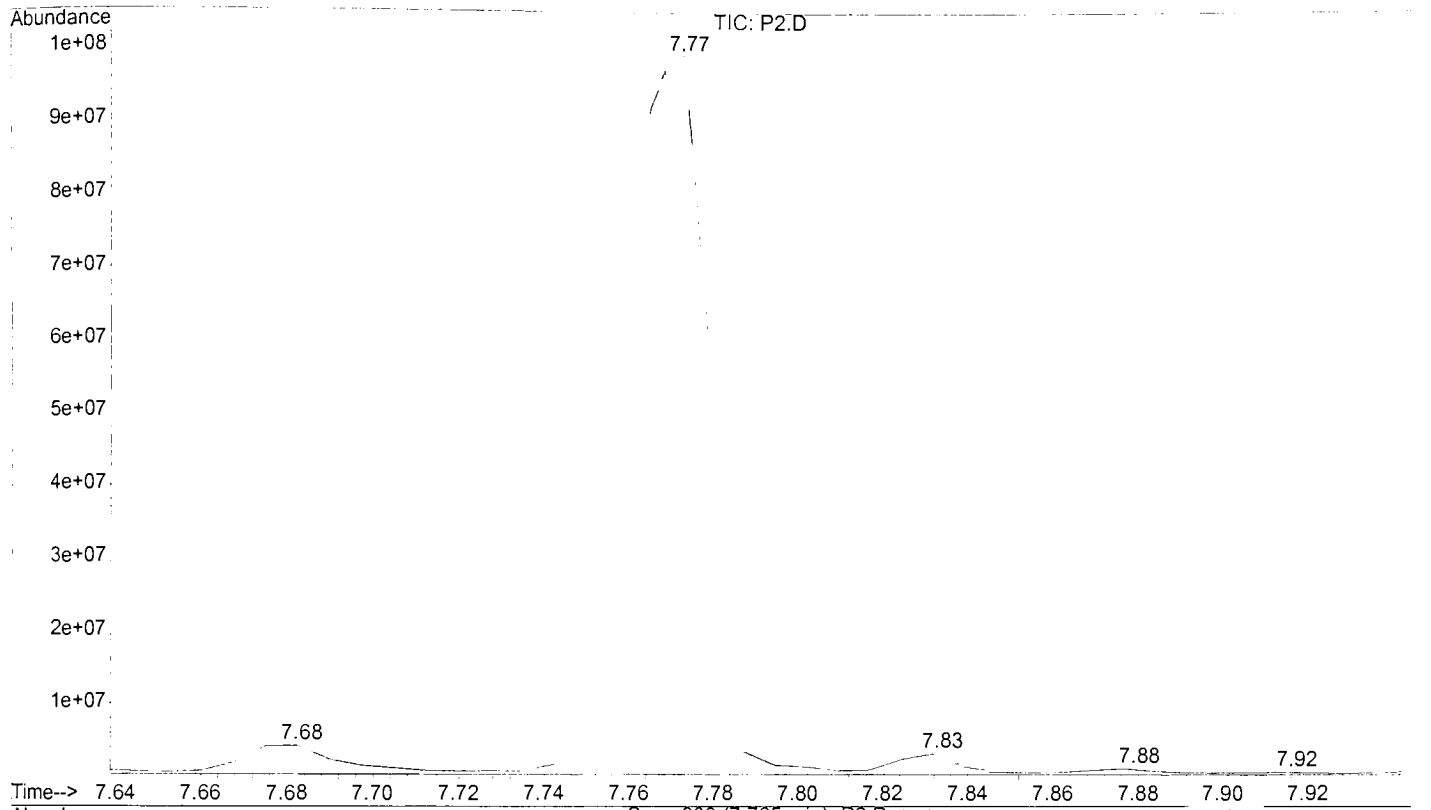
le : C:\HPCHEM\1\DATA\P2.D
erator : Pankaj
quired : 4 Jan 2000 14:47 using AcqMethod PANKAJ
strument : RIT Chemi
mple Name: Aniline, 4- bromo- N,N - dimethyl-
sc Info :
al Number: 1

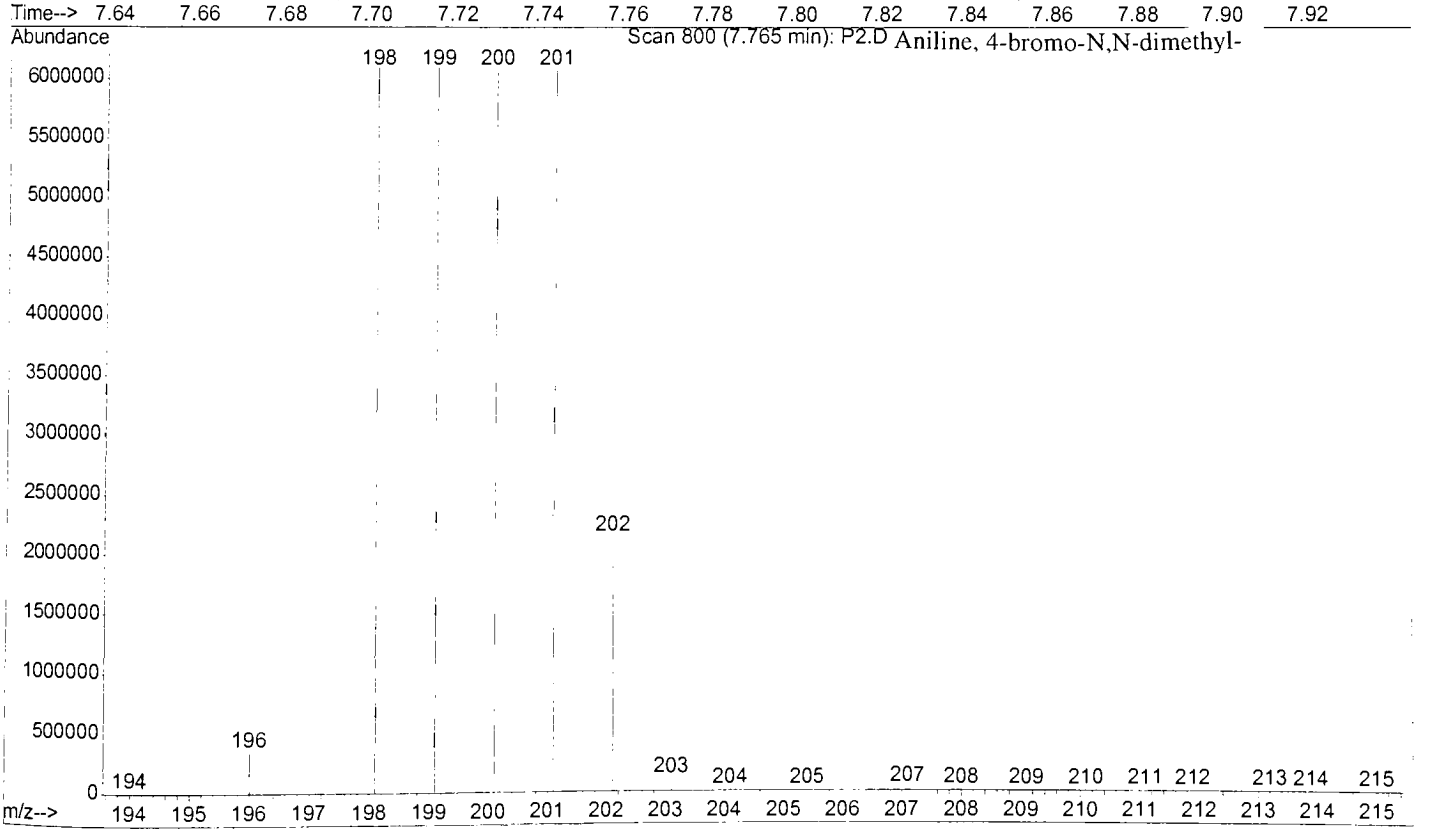
MS Spectrum 6.





.le : C:\HPCHEM\1\DATA\P2.D
)erator : Pankaj
:quired : 4 Jan 2000 14:47 using AcqMethod PANKAJ
ıstrument : RIT Chemi
ımple Name: Aniline, 4- bromo- N,N - dimethyl- MS Spectrum 6.
.sc Info :
.al Number: 1



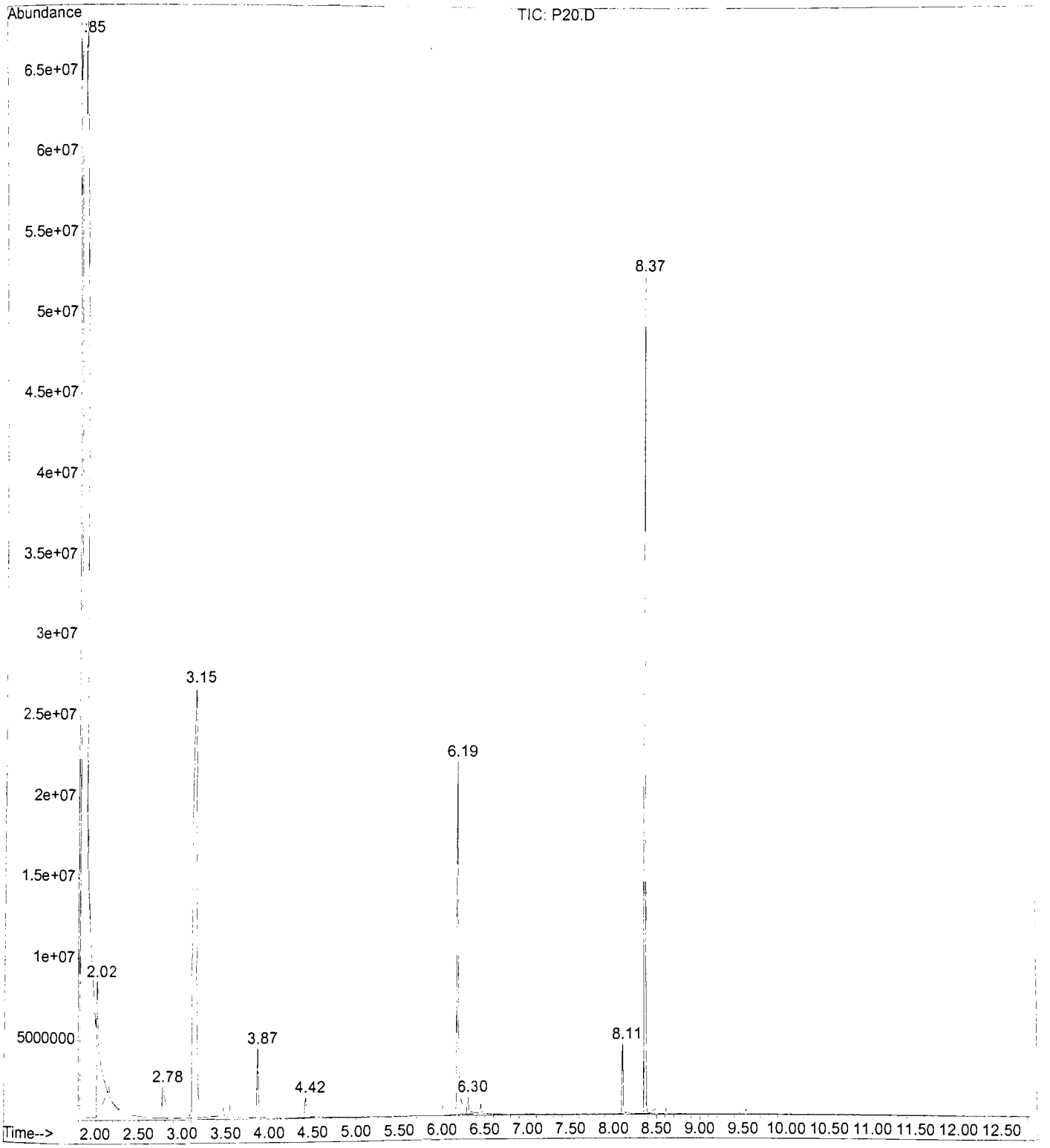


GC Spectrum 7. Area Percent Report

Data File : C:\HPCHEM\1\DATA\P20.D
Acq On : 9 Mar 2000 10:42
Sample : phenol+hcl
Misc :

Vial: 1
Operator:
Inst : RIT Chemi
Multiplr: 1.00
Sample Amount: 0.00

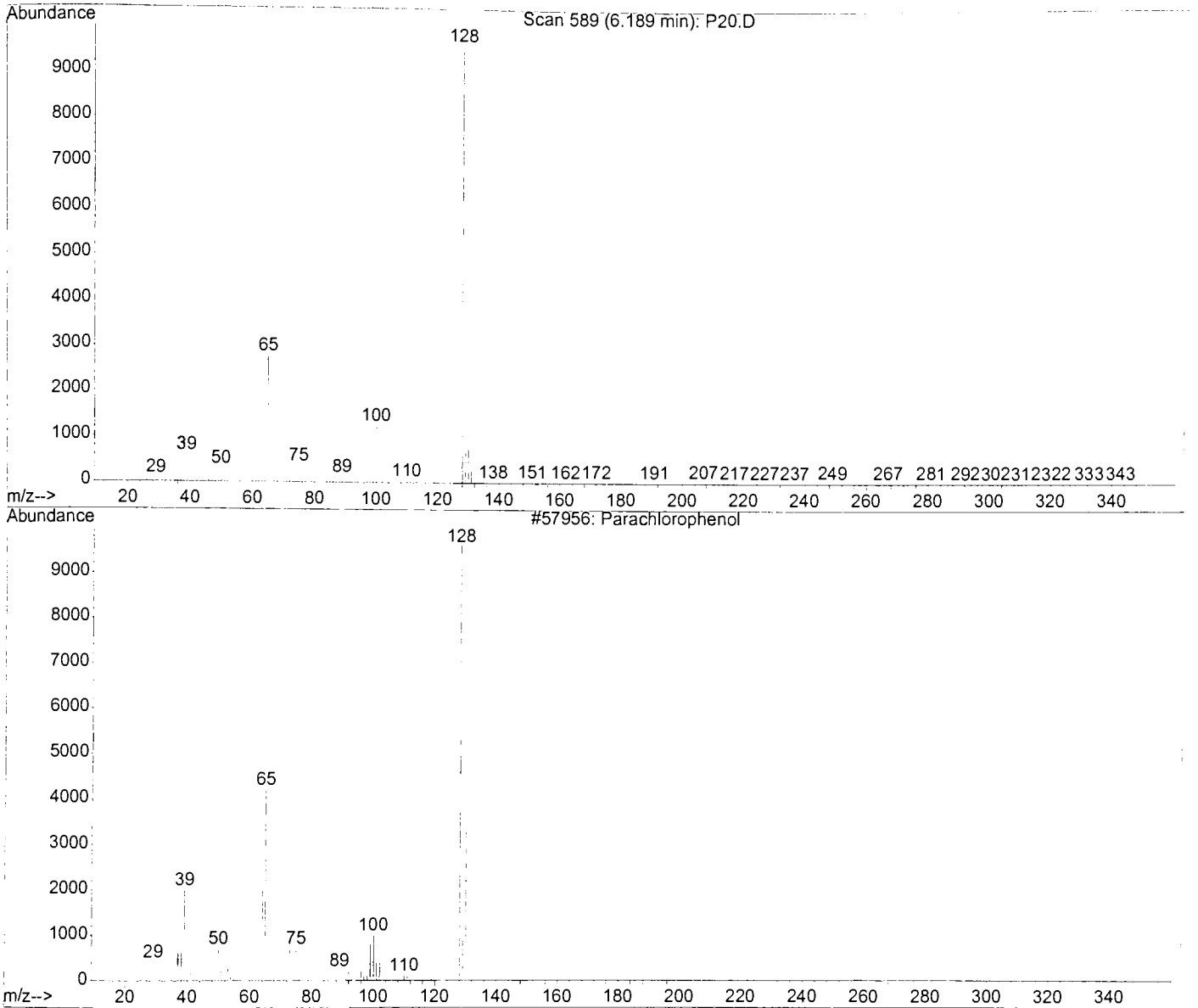
MS Integration Params: autoint1.e
Method : C:\HPCHEM\1\5973\PANKAJ.M (Chemstation Integrator)
Title :



P20.D PANKAJ.M Thu Mar 09 10:55:26 2000

Library Searched : C:\DATABASE\NIST98.L
Quality : 94
ID : Parachlorophenol

MS Spectrum 7.

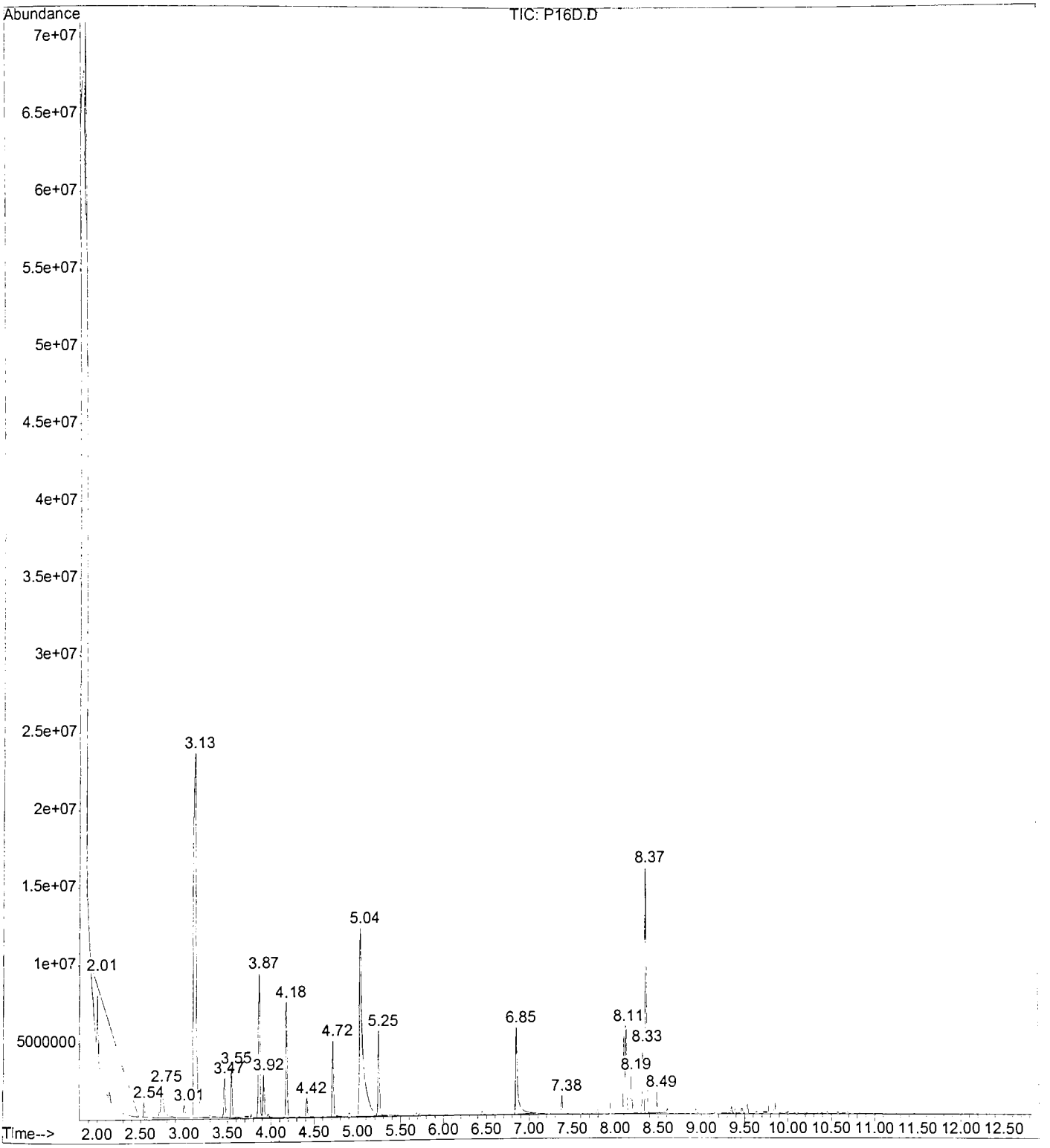


GC Spectrum 8. Area Percent Report

Data File : C:\HPCHEM\1\DATA\P16D.D
Acq On : 9 Mar 2000 11:12
Sample : phenol++++hbr
Misc :

Vial: 1
Operator:
Inst : RIT Chemi
Multiplr: 1.00
Sample Amount: 0.00

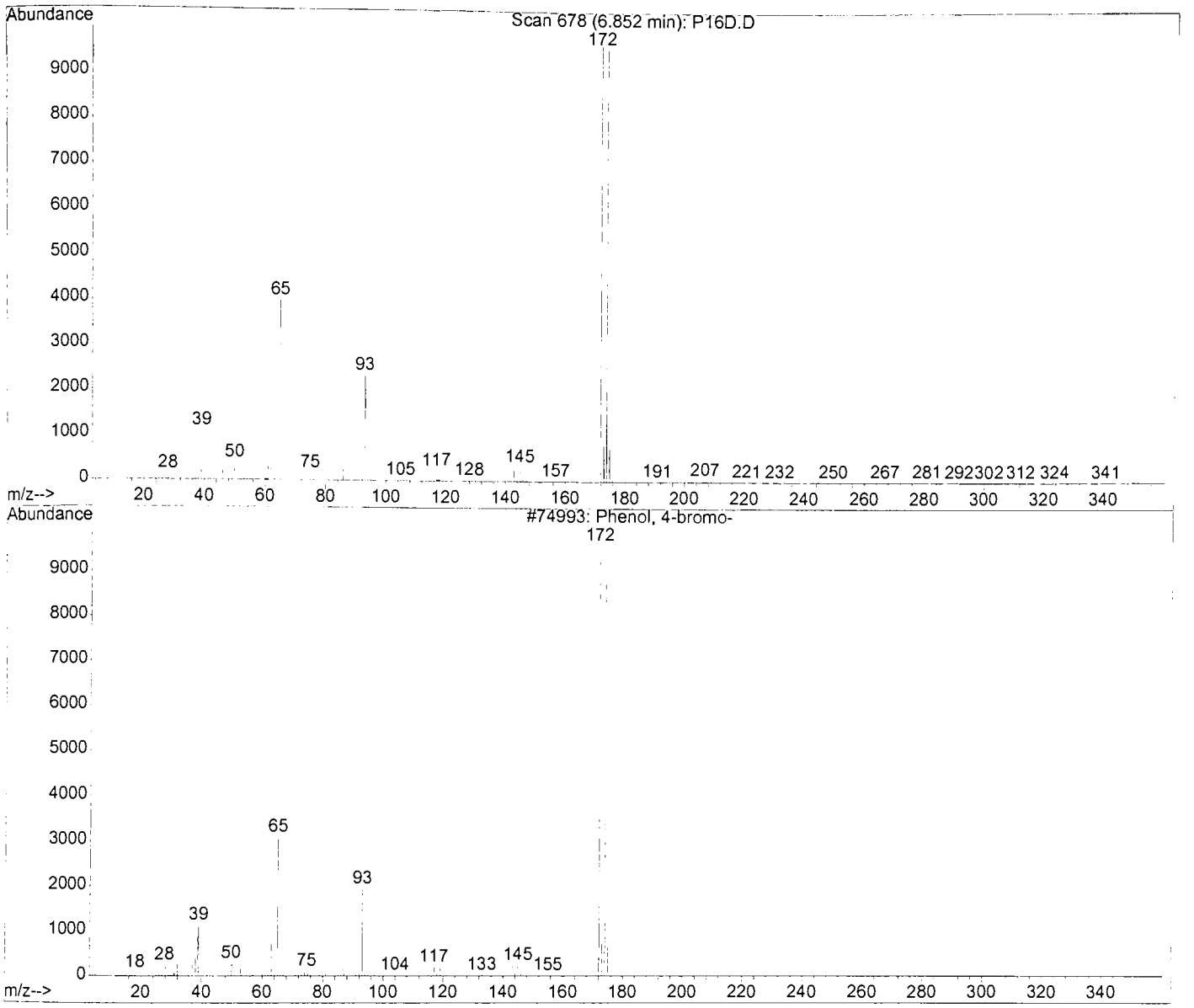
MS Integration Params: autoint1.e
Method : C:\HPCHEM\1\5973\PANKAJ.M (Chemstation Integrator)
Title :



P16D.D PANKAJ.M Thu Mar 09 11:25:50 2000

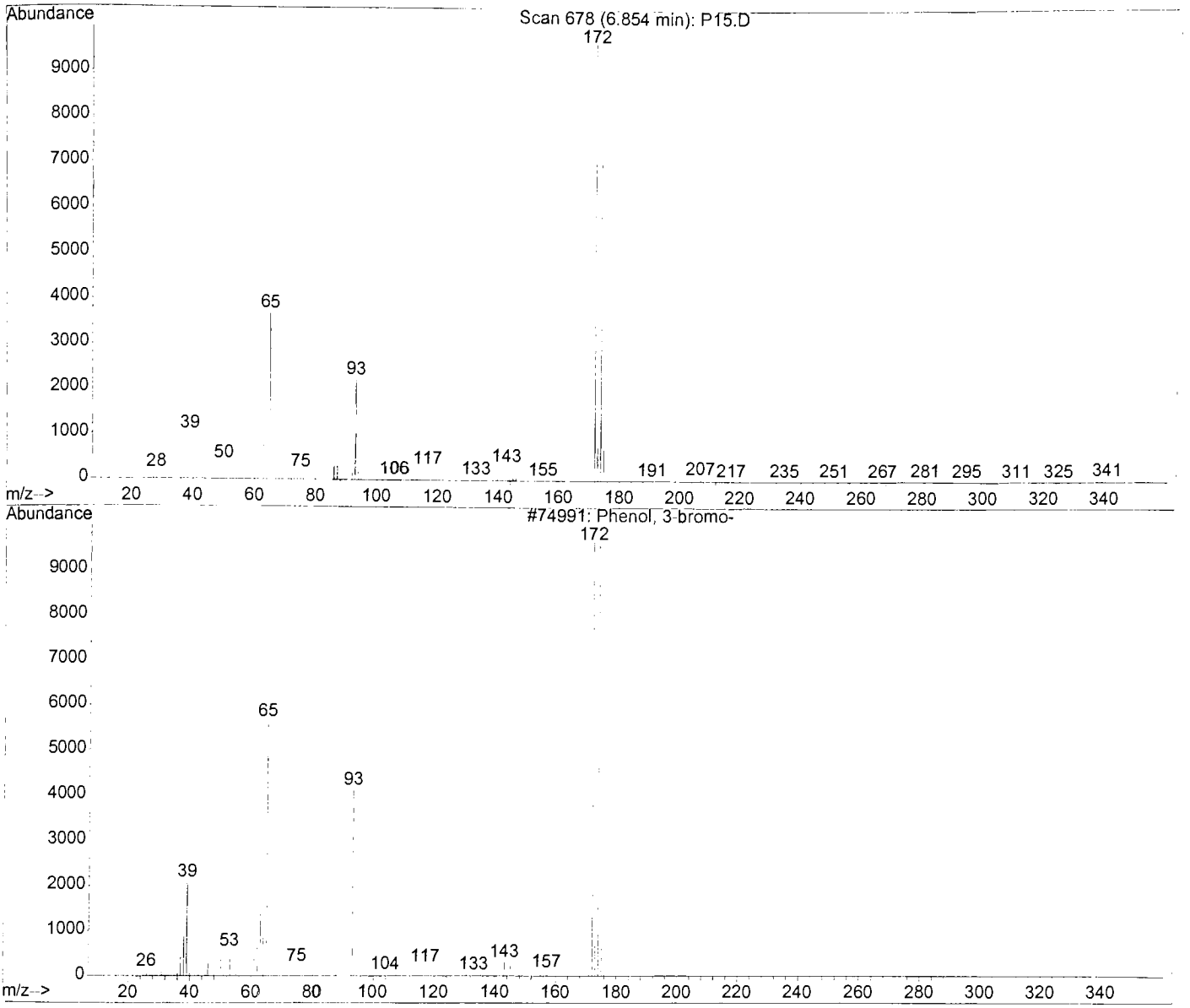
Library Searched : C:\DATABASE\NIST98.L
Quality : 94
ID : Phenol, 4-bromo-

MS Spectrum 8.



Library Searched : C:\DATABASE\NIST98.L
Quality : 94
ID : Phenol, 3-bromo-

MS Spectrum 8.

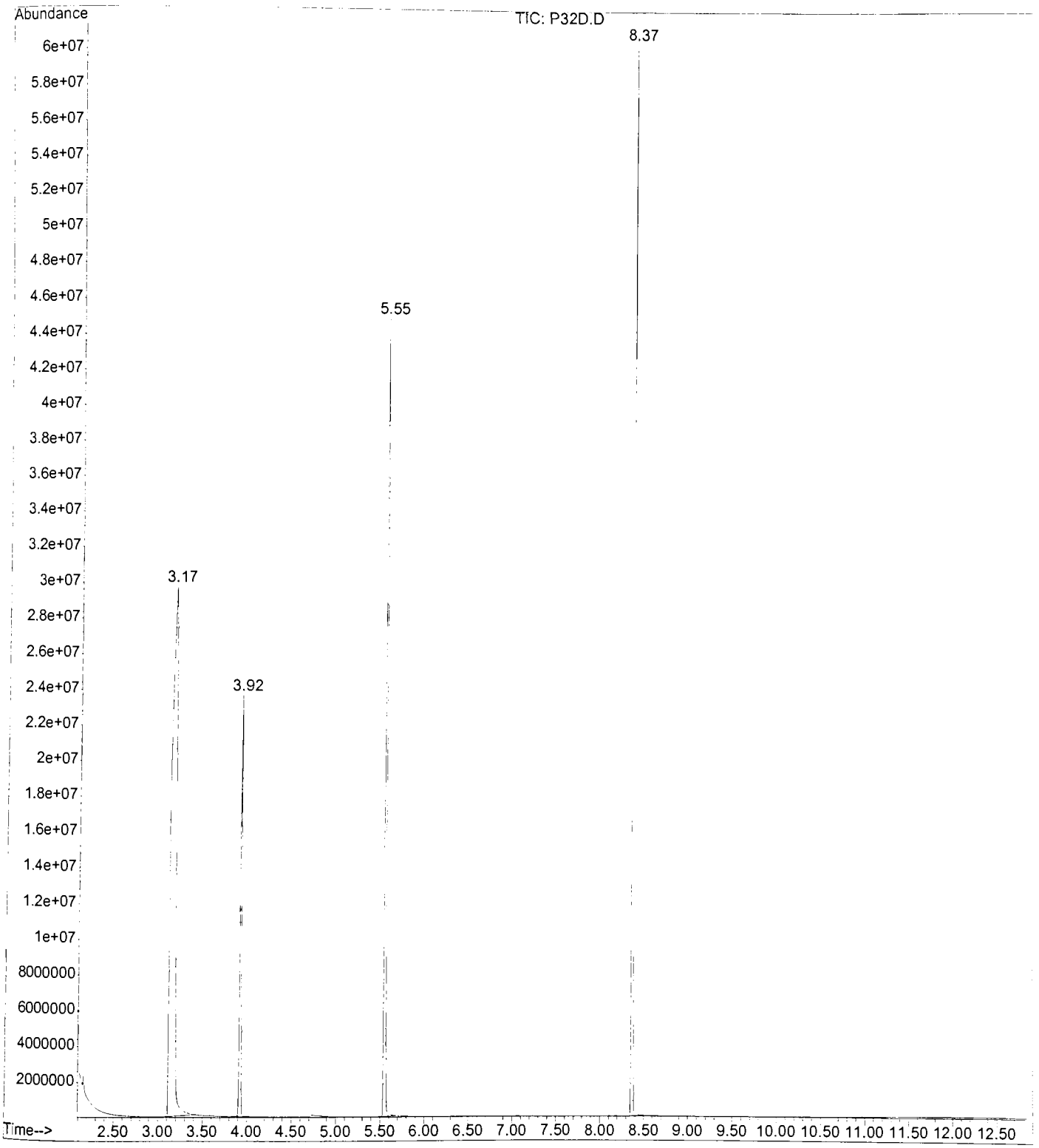


GC Spectrum 9. Area Percent Report

Data File : C:\HPCHEM\1\DATA\P32D.D
Acq On : 9 Mar 2000 15:23
Sample : anisole++hcl no.2
Misc :

Vial: 1 B9
Operator:
Inst : RIT Chemi
Multiplr: 1.00
Sample Amount: 0.00

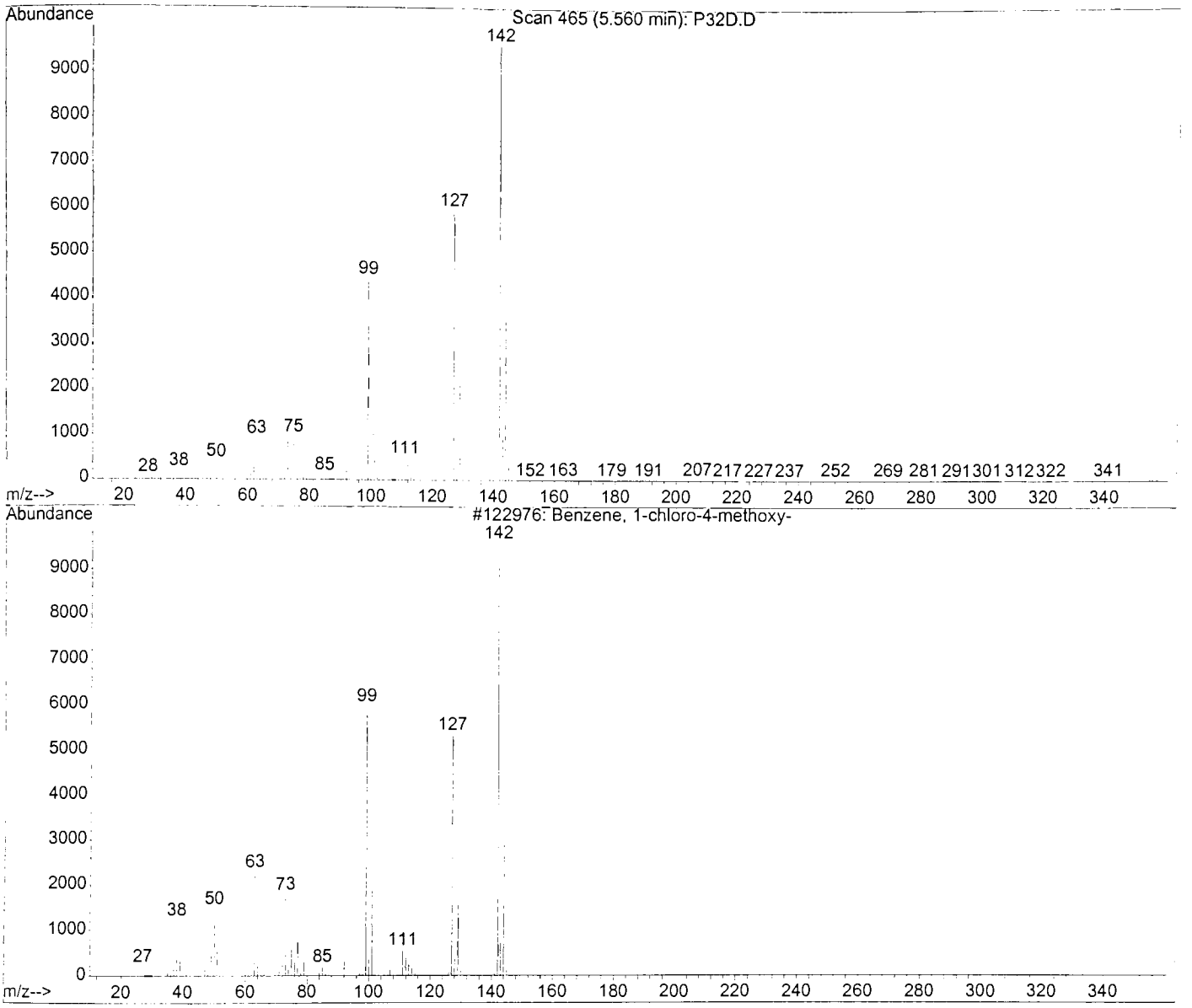
MS Integration Params: autoint1.e
Method : C:\HPCHEM\1\5973\PANKAJ.M (Chemstation Integrator)
Title :



P32D.D PANKAJ.M Thu Mar 09 15:36:00 2000

Library Searched : C:\DATABASE\NIST98.L
Quality : 95
ID : Benzene, 1-chloro-4-methoxy-

MS Spectrum 9.

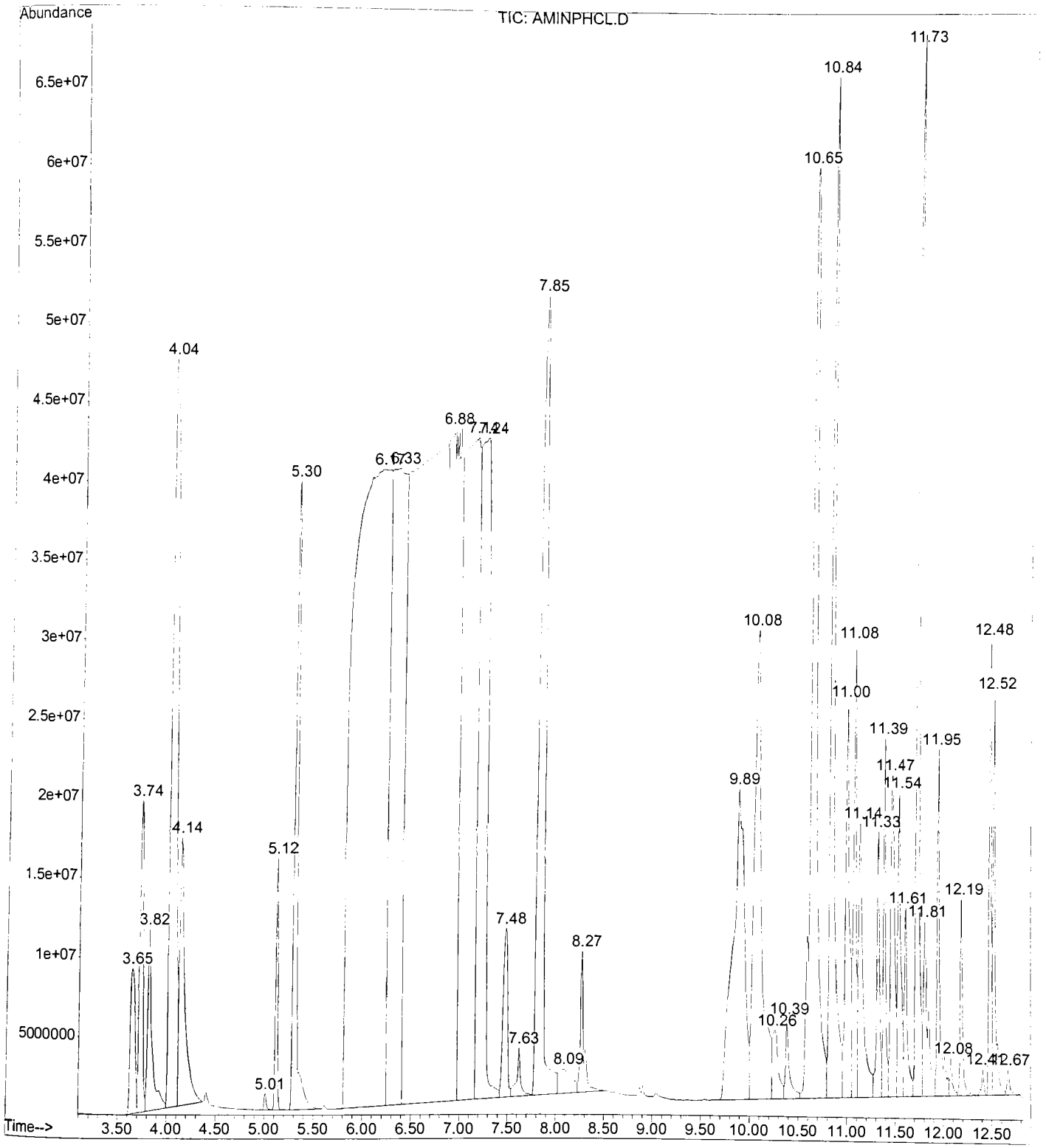


GC Spectrum 10. Area Percent Report

Data File : C:\HPCHEM\1\DATA\AMINPHCL.D
Acq On : 13 Jul 2000 20:10
Sample :
Misc :

Vial: 1 B10
Operator:
Inst : RIT Chemi
Multiplr: 1.00
Sample Amount: 0.00

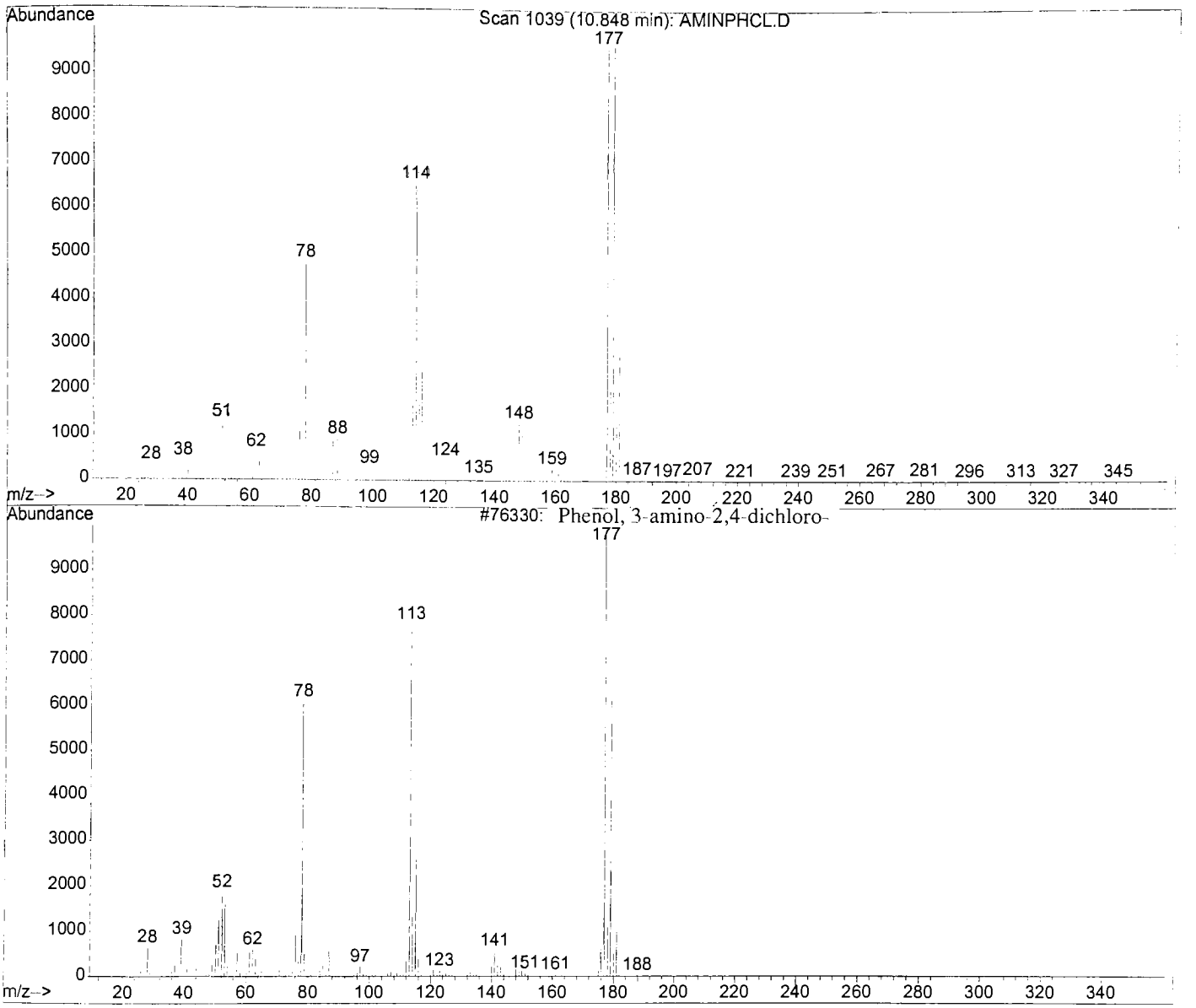
MS Integration Params: autoint1.e
Method : C:\HPCHEM\1\5973\PANKAJ.M (Chemstation Integrator)
Title :



AMINPHCL.D PANKAJ.M Thu Jul 13 20:23:04 2000

Library Searched : C:\DATABASE\NIST98.L
Quality : 42
ID : Phenol, 3-amino-2,4-dichloro-

MS Spectrum 10.

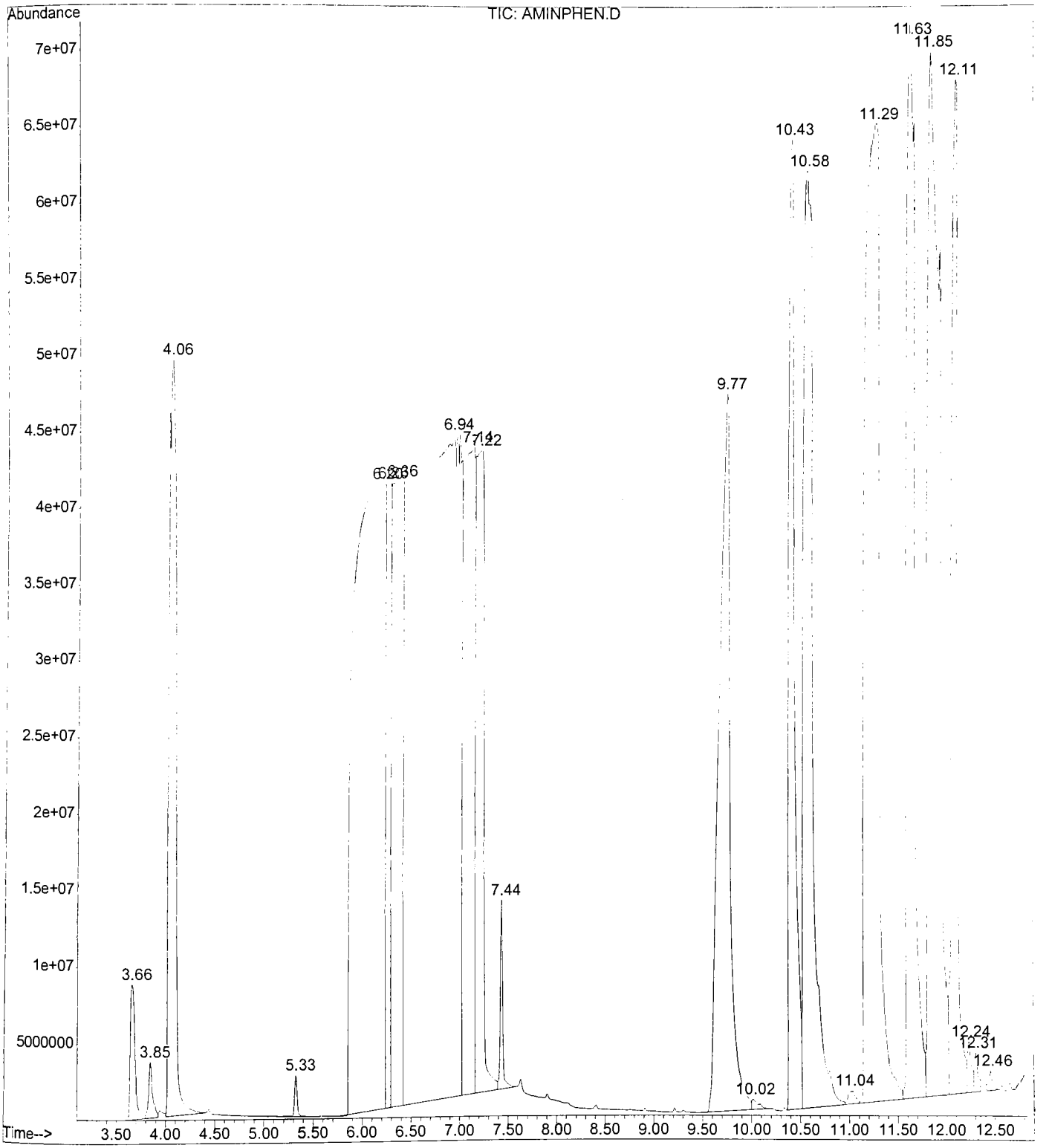


GC Spectrum 11. Area Percent Report

Data File : C:\HPCHEM\1\DATA\AMINPHEN.D
Acq On : 13 Jul 2000 19:43
Sample :
Misc :

Vial: 1 B11
Operator:
Inst : RIT Chemi
Multiplr: 1.00
Sample Amount: 0.00

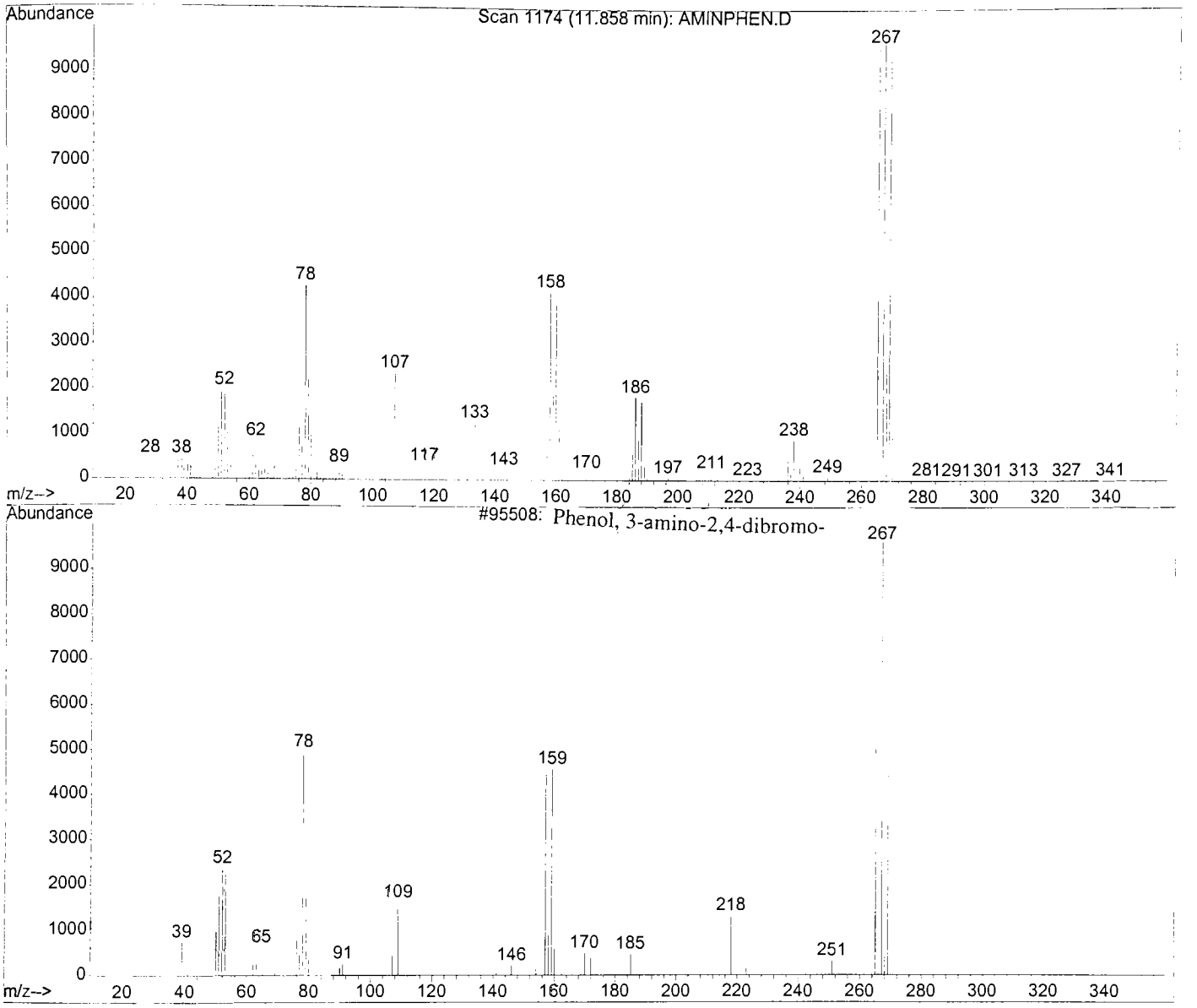
MS Integration Params: autoint1.e
Method : C:\HPCHEM\1\5973\PANKAJ.M (Chemstation Integrator)
Title :



AMINPHEN.D PANKAJ.M Thu Jul 13 19:55:56 2000

Library Searched : C:\DATABASE\NIST98.L
Quality : 25
ID : Phenol, 3-amino-2,4-dibromo-

MS Spectrum 11.

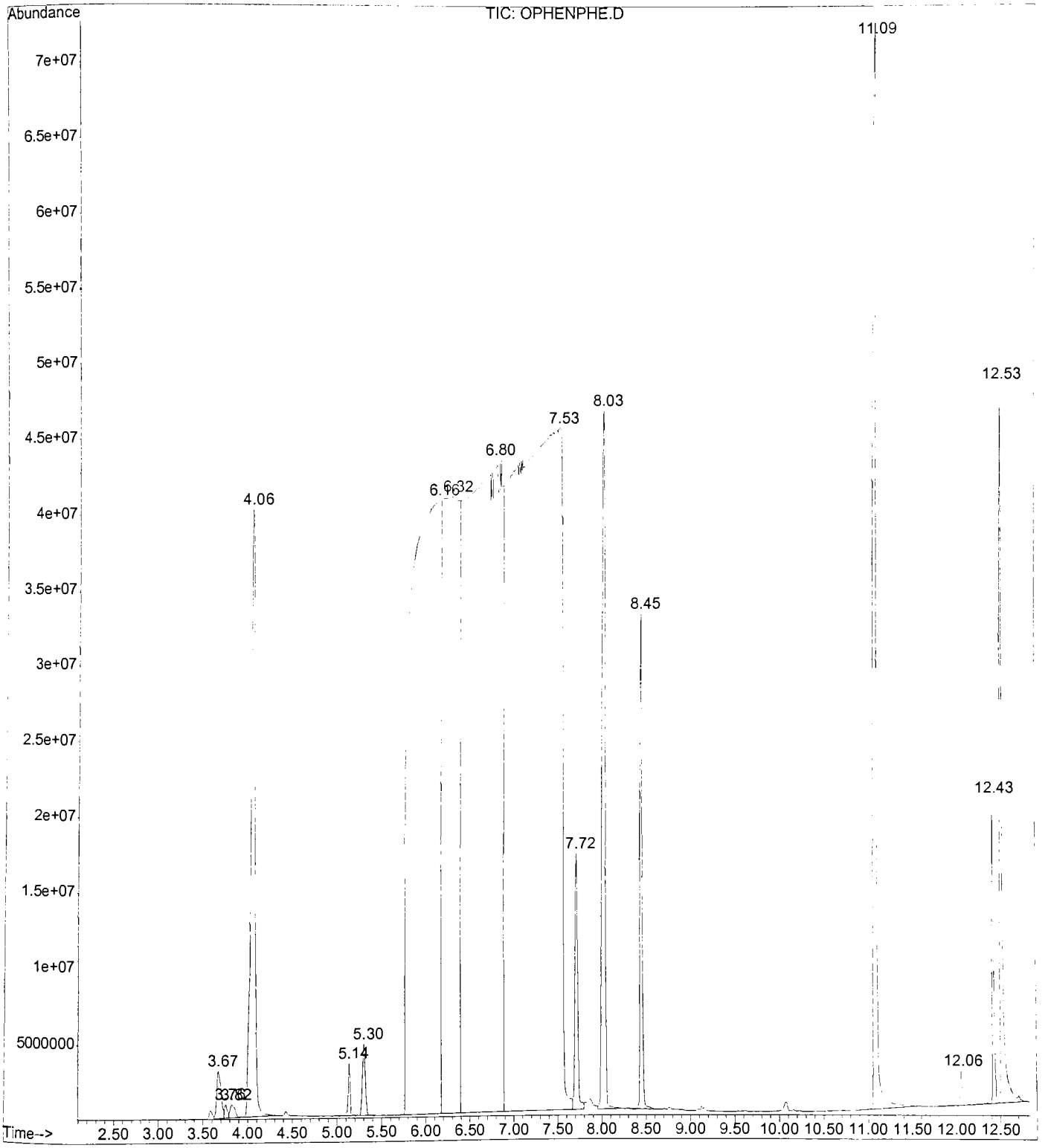


GC Spectrum 12. Area Percent Report

Data File : C:\HPCHEM\1\DATA\OPHENPHE.D
Acq On : 21 Jun 2000 16:16
Sample :
Misc :

Vial: 1 B12
Operator:
Inst : RIT Chemi
Multiplr: 1.00
Sample Amount: 0.00

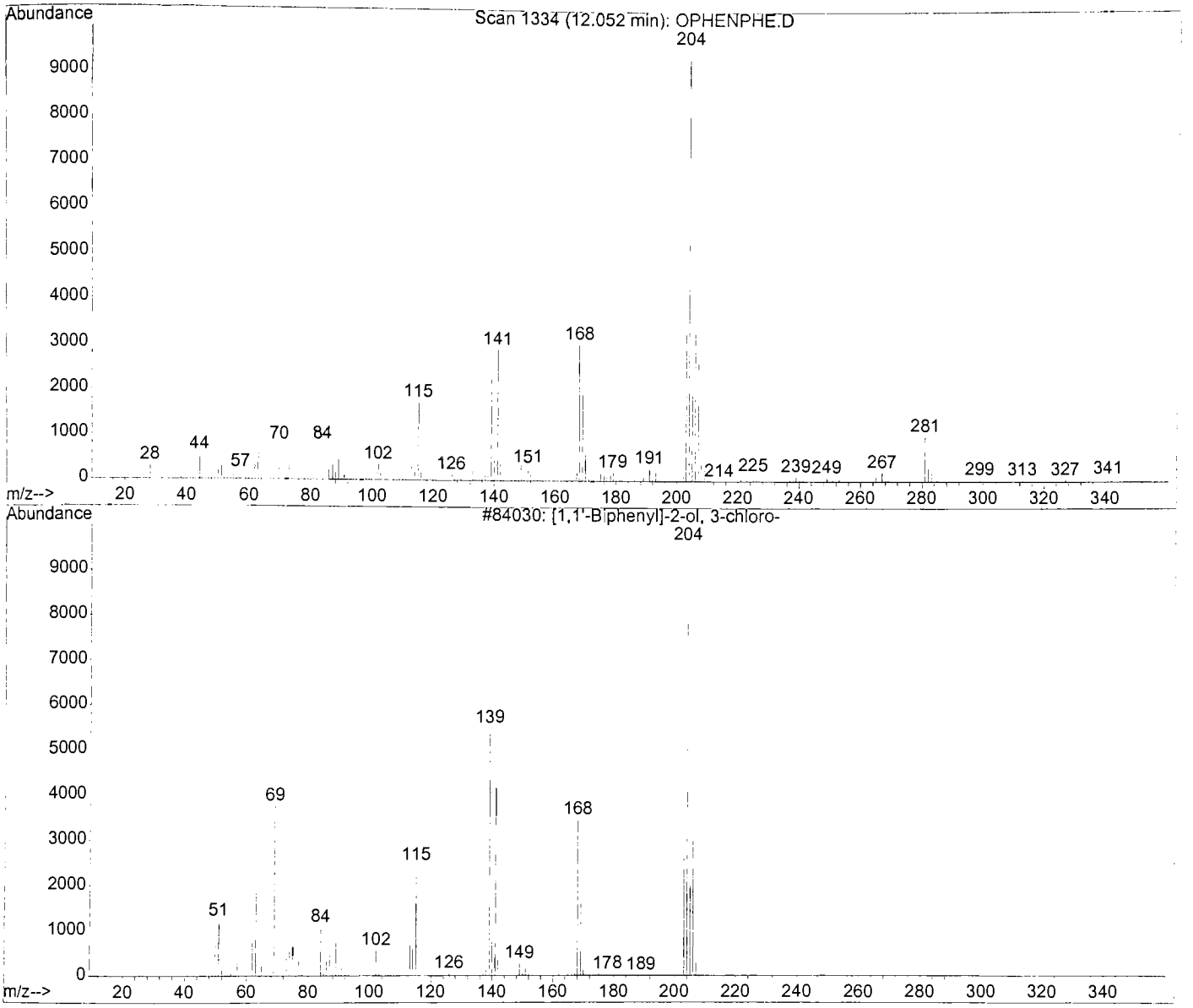
MS Integration Params: autoint1.e
Method : C:\HPCHEM\1\5973\PANKAJ.M (Chemstation Integrator)
Title :



OPHENPHE.D PANKAJ.M Wed Jun 21 16:29:35 2000

Library Searched : C:\DATABASE\NIST98.L
Quality : 90
ID : [1,1'-Biphenyl]-2-ol, 3-chloro-

MS Spectrum 12.



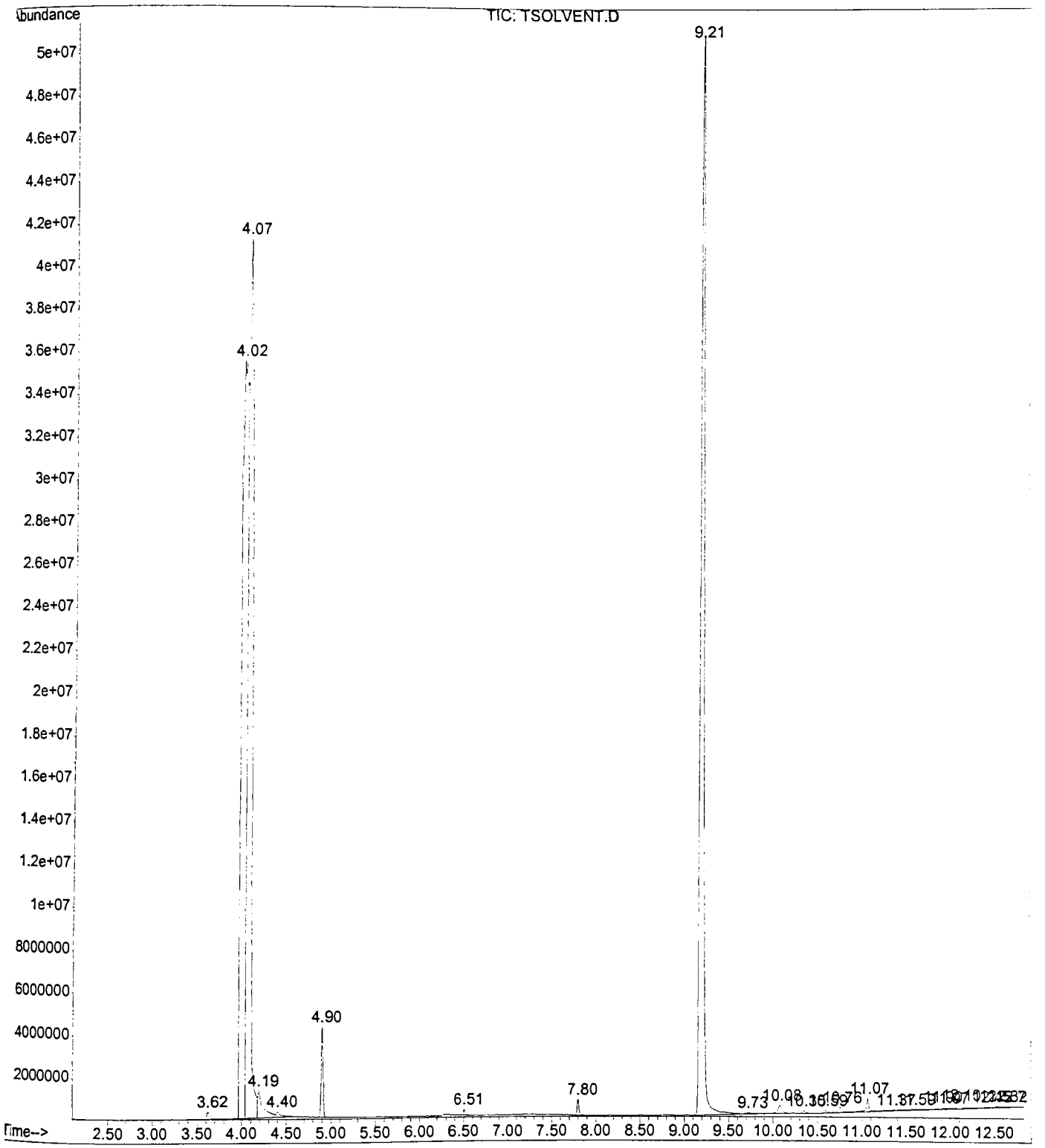
GC Spectrum 13. Area Percent Report

B13

Data File : C:\HPCHEM\1\DATA\OPHENHBR.D
Acq On : 21 Jun 2000 16:57
Sample :
Misc :

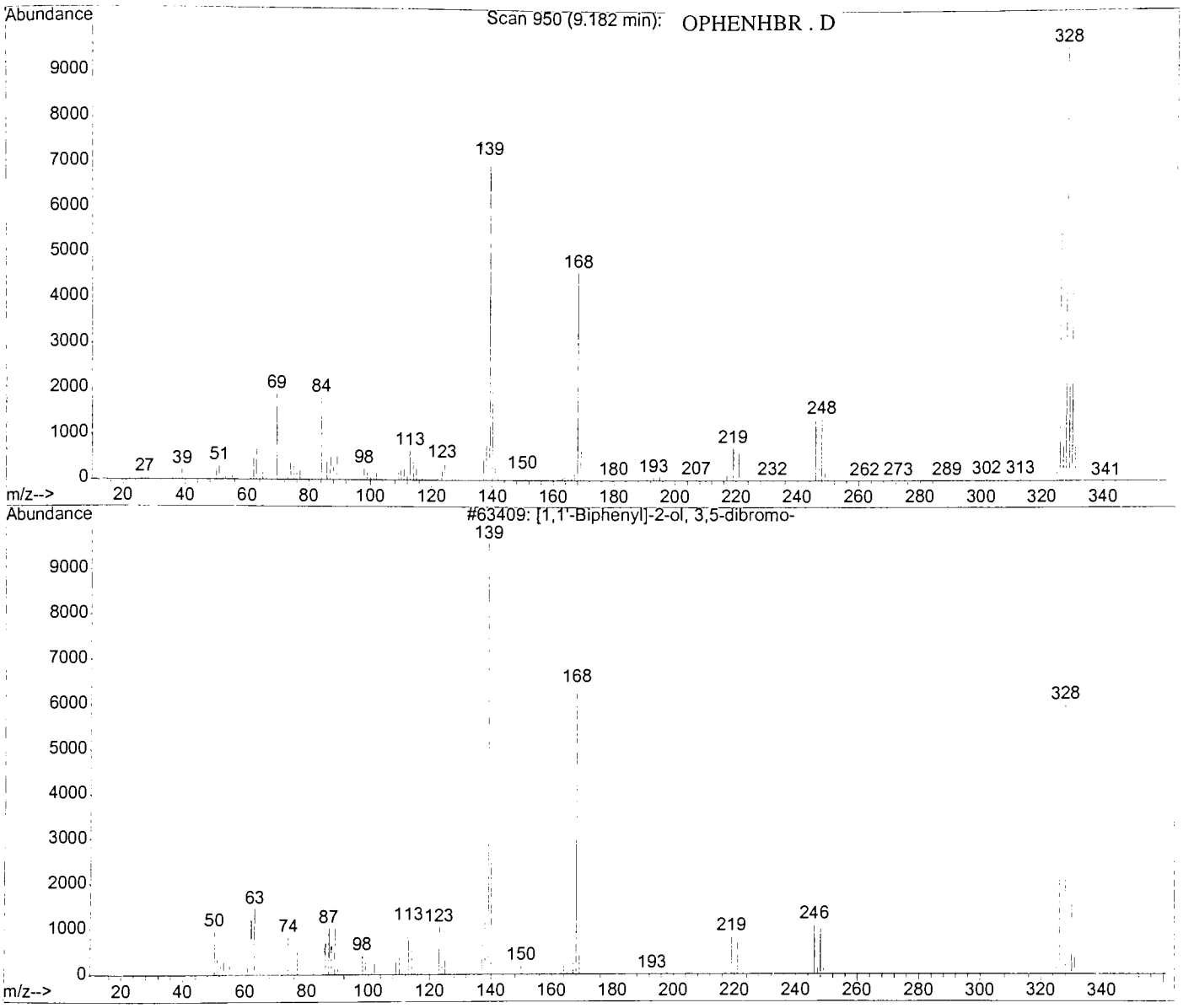
Vial: 1
Operator:
Inst : RIT Chemi
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e
Method : C:\HPCHEM\1\5973\TRACY.M (Chemstation Integrator)
Title :



TSOLVENT.D TRACY.M Wed Jun 21 17:10:24 2000

Library Searched : C:\DATABASE\NIST98.L
Quality : 95
ID : [1,1'-Biphenyl]-2-ol, 3,5-dibromo- MS Spectrum 13.



APPENDIX C

Table of NMR Parameters

Current Data Parameters		
NAME	Jun19-00	
EXPNO	6	
PROCNO	1	
F2 - Acquisition Parameters		
Date_	500000	
Time	16.51	
INSTRUM	spect	
PROBHD	5 mm QNP 1H	
PULPROG	zg30	
TD	32768	
SOLVENT	DMSO	
NS	16	
DS	2	
SWH	6172.839	Hz
FIDRES	0.188380	Hz
AQ	2.6542580	sec
RG	28.5	
DW	81.000	usec
DE	6.00	usec
TE	300.0	K
D1	1.00000000	sec
P1	9.00	usec
DE	6.00	usec
SFO1	300.1318534	MHz
NUC1	1H	
PL1	-5.00	dB
F2 - Processing parameters		
SI	16384	
SF	300.1300000	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	
1D NMR plot parameters		
CX	20.00	cm
F1P	11.000	ppm
F1	3301.43	Hz
F2P	-1.000	ppm
F2	-300.13	Hz
PPMCM	0.60000	ppm/cm
HZCM	180.07800	Hz/cm

APPENDIX D

Data Analysis Parameters

Method Name: C:\HPCHEM\1\5973\CHRIS.M

Percent Report Settings

Sort By: Retention Time

Output Destination
    Screen: Yes
    Printer: Yes
    File: No

Integration Events: AutoIntegrate

Generate Report During Run Method: Yes

Signal Correlation Window: 0.020

Qualitative Report Settings

Peak Location of Unknown: Apex

Library to Search	Minimum Quality
C:\DATABASE\NIST98.L	0

Integration Events: AutoIntegrate

Report Type: Summary

Output Destination
    Screen: No
    Printer: Yes
    File: No

Generate Report During Run Method: Yes

Quantitative Report Settings

Report Type: Summary

Output Destination
    Screen: Yes
    Printer: No

Calibration Last Updated: Mon Nov 20 15:48:49 1995

Reference Window: 10.00 Percent
Non-Reference Window: 5.00 Percent
Correlation Window: 0.02 minutes
Default Multiplier: 1.00
Default Sample Concentration: 0.00

Compound Information

---

1) mass 284 ( )

Ret. Time 7.28 min., Extract & Integrate from 6.78 to 7.78 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 283.90			*** AUTO ***

Lvl	ID	Conc ( )	Response
3		1.000	371748
4		10.000	3849624
5		100.000	18762380
2		0.100	31150

Qualifier Peak Analysis ON
Curve Fit: Avg. RF

---

2) mass 283 ( )

Ret. Time 7.28 min., Extract & Integrate from 6.78 to 7.78 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 283.90			*** AUTO ***

Lvl	ID	Conc ( )	Response
3		1.000	371748
4		10.000	3849624
5		100.000	18762380
2		0.100	31150

Qualifier Peak Analysis ON
Curve Fit: Avg. RF

---

3) mass 247 ( )

Ret. Time 7.28 min., Extract & Integrate from 6.78 to 7.78 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 246.90			*** AUTO ***

1.000	44562
10.000	479738
100.000	2662997
0.100	-1

.alifier Peak Analysis ON
.rve Fit: Avg. RF

:) mass 212 ( )

:t. Time 7.28 min., Extract & Integrate from 6.78 to 7.78 min.

.gnal	Rel Resp.	Pct. Unc.(rel)	Integration
gt 212.00			*** AUTO ***

rl ID	Conc ( )	Response
	1.000	25707
	10.000	264918
	100.000	1532237
	0.100	-1

ıalifier Peak Analysis ON
ırve Fit: Avg. RF

END OF DATA ANALYSIS PARAMETERS

Thu Jul 13 15:56:22 2000